

nature

2 November 1978

Plenty for GMAG to do

LAST week ASTMS, the Association of Scientific Technical and Managerial Staffs, held a one-day meeting to discuss the social and safety implications of genetic engineering. Some of the more scientific aspects of the meeting are reported on the following pages, but an equal amount of time was devoted to the politics of monitoring scientific research, with particular reference to GMAG, the Genetic Manipulation Advisory Group to which the British government has delegated responsibility for giving advice on precautions to be taken in research on recombinant DNA. GMAG is itself an interesting experiment, as the eight working scientists are in a minority on it, and there are four representatives of 'the public interest' and four nominated by the Trades Union Congress, of which ASTMS provides two.

Naturally ASTMS's motives in convening such a meeting were not entirely free of self-interest. As a vigorous, expanding white-collar union it was seeking to impress not just its own members but the world at large with its deep concern over health and safety and warn the nascent biological industry of its intention of going on to the attack in this area. Whether it succeeded is a moot point. The barn-storming talk about 'confrontation situations' and the caricatures of industrial and university management no doubt pleased the ASTMS rank and file who turned out in good numbers, but those research scientists not associated with the union (university teachers, for instance) might well have wondered whether in all the strong words the peculiar needs of universities, and even more of science itself, for freedom of action were in danger of being assigned too much of a back seat. They might also have been somewhat uneasy at what exactly was meant by the talk of trades unions getting more involved in the making of science policy, and this is something which Clive Jenkins, general secretary of the union, would do well to expand on in the near future.

It was illuminating or confusing, depending on one's point of view, that the Birmingham smallpox tragedy was still fresh in everyone's mind. Illuminating to those who believe that the Dangerous Pathogens Advisory Group (DPAG), comprised only of scientific experts, is ripe for reform along GMAG lines, and that the Birmingham case shows only too clearly that scientists cannot be allowed to go on monitoring themselves. Confusing to those who, having come to a meeting, as they thought, on GMAG, felt the question of dangerous pathogens only marginally relevant and one step along the slippery path that ends up with recombinant DNA, germ warfare and test-tube babies all being lumped together in the public's mind. It was also useful to ASTMS, who could connect the passions raised by the Birmingham affair with the more imponderable matters of genetic manipulation.

What of GMAG itself? It is now approaching its second anniversary, and the members of it who spoke, including Sir

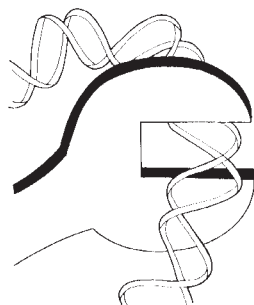
Gordon Wolstenholme, its chairman, were clearly proud of how it worked and what it had achieved. It was spoken of as a model for public and union intervention in matters of widespread concern, and one of its members even described it, in the conference's accompanying papers, as having been accomplished "with customary British decorum and discretion . . . a model for all the world". Rightly did several people point to the importance of representative laboratory safety committees as a cornerstone of GMAG's activities. And yet, for all the enthusiasm, there remain question-marks.

One is the problem of industry, which is moving rapidly into genetic engineering. ASTMS failed to get a large representation of industrial directors and managers at the meeting—not for want of trying, the union claimed. One industrialist who did come out and speak, Dr James Combes of Hoechst, made it clear that industry viewed the restrictions on genetic manipulation as oppressive. He would have liked a GMAG that was simply a technical committee (which would inevitably rest on an uneasy separation of facts from value judgments), and he voiced concern over confidentiality and the question of revealing industrial secrets to GMAG. To some of those present, talk of patents and industrial confidentiality was, of course, anathema. And yet, if industrialists, unionists and research scientists cannot enter into a reasoned dialogue over their hopes and needs, the future for the British biological industry will not be rosy. Perhaps this meeting provided a tentative first step.

A second question mark is over GMAG's external relations. Sir Gordon spoke of the strong group loyalty which had emerged amongst members who are, in any case, bound (by having signed the Official Secrets Act) not to reveal details of their work. But this very laudable loyalty can militate against outsiders being allowed to share in GMAG's thinking. Scientists contemplating certain experiments are expected to bring them to GMAG, but problems GMAG itself faces—these are not to be shared.

As it happened there was a case in point at the meeting. All GMAG members knew, and several others present did also, that the thinking behind Dr Sydney Brenner's talk has already been enshrined in a completely new set of draft criteria for risk-assessment to be considered by GMAG in a matter of days. Clearly this meeting was not the place for the new material to be discussed in detail. Yet it is a matter of some surprise that not a single word was said about them. Certainly a committee as large as GMAG leaks, but surely open government is not best done by leaks, however judicious. GMAG is required to issue an annual report. It would be good if it could also be required to hold public meetings, not less frequently than every six months, to talk about the difficulties it faces and to hear views from industry, academe, government laboratories and the unions. The ASTMS meeting was a start. Let it be followed up. □





LAST WEEK, the British trade union, the Association of Scientific, Technical, and Managerial Staffs (ASTMS), called the first public debate in Britain on the potential hazards of genetic manipulation. In the end there was little debate, which may indicate the level of public understanding of the question, but there were stimulating contributions from the platform. Here we reproduce shortened versions of the talks of Sydney Brenner, director-designate of the Medical Research Council's unit of molecular biology at Cambridge, and Jonathan King, Professor of Biology at the Massachusetts Institute of Technology. On a following page Eleanor Lawrence disentangles the threads of the biology involved, for the meeting blurred the distinc-

tion between genetic manipulation proper (using restriction enzymes *in vitro*) and other work (such as the investigation of dangerous pathogens, or recombination in natural systems). Finally, an ASTMS officer explains her union's plans to radicalise university research laboratories in Britain. TODAY Britain's Genetic Manipulation Advisory Group (GMAG) is considering whether to adopt a new set of principles for assessing the hazards of individual experiments, much more precise than the 'rule of thumb' phylogenetic system so far adopted in the UK, the US, and elsewhere. Sydney Brenner was its inventor.

NEXT WEEK, if GMAG adopts the new guideline, *Nature* will publish a condensed version of them.

Six months in category four

By Sydney Brenner

I've been associated with this debate almost from its inception; and I can say that I have given a considerable amount of my time—perhaps four years of my life—to it, and certainly something like ten shelves in my office to the paper that has flowed out of the discussions. These discussions have been very extensive. It will be impossible here to go into all of the details of the subject. But one must look at the historical constraints under which the subject was approached.

Interest in the subject of 'genetic engineering' or 'genetic manipulation' was first aroused by a letter written by some Americans—Paul Berg and others—requesting a moratorium on this research. I must tell you that there was an immediate response in this country. I think that whereas the moratorium was enforced in this country, it was only treated as a voluntary matter in other countries. As an employee of the Medical Research Council (MRC) I received a letter at the time, from the then head of the organisation, instructing me to obey the moratorium. Of course it was quite easy to obey because we weren't doing experiments.

The Ashby Committee in the UK, followed by meetings at Asilomar, which was international, followed by the Williams working party, resulted in the institution of the Genetic Manipulation Advisory Group (GMAG). And I think that it is a unique institution in the world, in that it involves formally the participation of representatives of organisations who are not scientists. And insofar as the trades unions are part of the public, one has good public input.

Now why have we had this very elaborate organisation, GMAG? I think it is very important to understand why this particular area has been singled out for very special attention. It has been singled out for special attention because of the statement made



"We 'genetic manipulators' question why this area has been singled out."

very early that there were particular dangers associated with genetic manipulation—that is, risks that were so particular, and potentially so great, that people had better start to take special action about them.

That created the historical background for why we have a GMAG for genetic manipulation. But there are lots of other things you can do in laboratories over which there is no control at all. Among we 'genetic manipulators' (if such a profession exists) there is a questioning of why this area has been singled out. It is the perception of people in the field that right next door there are people who do much worse things—you know, with the fags hanging out of the mouth, and a cup of tea, doing it any old way they like.

Now I think this is quite important, because one should desperately avoid the situation that one branch of science is singled out for the delivery of social punishment. Now I don't want to say this has happened, but I do want to emphasise that this is a kind of connection between science and society that we must avoid: that if scientists have been bad boys, as many people think—science is being questioned—then the genetic manipulators can so to speak carry the can and get six months in category four. That is a psychological situation that must be avoided.

We must begin to ask new questions about this whole area of research. One has heard the statement made this morning that a researcher can put antibiotic resistance into *Shigella sonnei* with no control; and you have to ask why isn't anybody controlling him? No one's controlling him because he is using natural mechanisms. That is, he's using the mechanisms used in nature to generate new strains—mutation, recombination, even perhaps genes that jump around from one piece of DNA to another; all he is applying to these mechanisms is special selection. He is fishing out of nature events that may be extremely rare and enriching them in a laboratory. His technology is that of enrichment. He creates by his techniques a local high concentration of such elements, of which there are enormous numbers, that lie all over the place.

What a genetic manipulator does is to do things *in vitro*. In theory, if you could do genetic manipulation by avoiding the use of test tubes then in fact you would be doing something that was a natural mechanism, and so in theory you might argue that you could escape from the GMAG regulations. I'm not offering that as a possibility, but I just want you to realise that the difference between genetic manipulation and other biology as understood by GMAG is that it is a sort of 'confined area' of genetic construction. And it is clear that you can make genetic constructions in organisms with natural mechanisms.

And so work on recombinant influenza viruses, work on recombinant bacteria, the transfer of promiscuous plasmids from one bacterium to another, that goes on, but doing the same work—indeed the identical experiment could be formulated—with a restriction enzyme would put you under the GMAG regulations. That is a paradoxical situation that we have to look into.

Why does one have to look into this? Because of the question of what constitutes a risk. There are now moves in the United States to change the so-called 'guidelines' (which may be looked on by some as attempts to amend the Ten Commandments); but the question is what are the grounds for these amendments? Now of course there is the important ground that we have new knowledge, but I think there is another ground which should be honestly stated: namely, that the risk analysis was not properly done. (This is my personal opinion). Now why wasn't it properly done?

First, there are things we can actually say are dangerous. It is a remarkable thing there are natural objects we can say are risky. Smallpox virus is risky. A lot of bacteria are risky. The people who work with them know they are risky, and they will therefore take precautions. You don't play games with smallpox. No one can say it is a fundamental belief of his that smallpox isn't dangerous. One can convince him very quickly it's dangerous. So there is immediate feedback to the workers involved.

So with these things there is no problem in declaring what is dangerous. We have—if you like—knowledge about that. We know from historical, clinical, and epidemiological evidence that certain things will knock you off with a high probability. And there seems to me to be no question that those should be declared as such, and if people are fools enough not to accept control on such work, then we will see events such as we have seen in the past—not only in this country but in other countries as well.

There are 5,000 recorded cases of laboratory infection in the United States, and some of them—with *rickettsia*—had fatal consequences. There's no doubt about this. I think it's on this sort of evidence that we have to rely to judge the effectiveness of physical containment. The experiments have been done for us in the past—alas they should not have been done as experiments, but they are there, and we have to rely on that information.

But how are we to assess the potential and certainly the conjectural risks? Who will say what is dangerous? The difficulty here in doing a risk assessment is that we are in a field where we are dealing not with nuclear reactors, not with toxic substances, but with self-replicating biological entities. We are far away from questions of linear dosage. In most of the other cases, chemical and other substances, one can make calculations quite clearly which are based on linear dosage relations. You know what the toxic dose is, or one can do experiments to establish it or estimate it quite accurately, and

therefore you can make conditions to prevent that toxic dose from reaching the workers and the public at large.

The trouble with the creation—or if I could call it the enhancement—of organisms is that they could depend very largely on their selective amplification. But that is the key thing. It is selective amplification that counts. Now let me make this clear what I mean. Years ago when I first started to travel in aeroplanes, with a very crude knowledge of physics and aerodynamics, I used to sit in those DC3s, and I had that marvellous vision with which I could look right into the aircraft engine; and I could see in all detail all the parts going round and round, the spinning of crankshafts and pistons and so on; and then I could actually see those hairline cracks developing. It used to worry me. I think many people look at genetic manipulation with that kind of internal vision. They see lying on the Petri dish the one horror bacterium, the one horror colony, the colony that is going to escape off that Petri dish and create global disaster.

I think that that feeling forms one of the most difficult hurdles to cross in trying to do objective risk assessment. And I believe that to be objective is very important. In the past in this field, we have had no more than the balance of example and counter-example. I have sat in on hundreds of arguments which have got into details that even mediaeval theologians could not have reached as to the total number of nucleotide base pairs that should be allowed under section B subsection A paragraph 1.2. And the situation has been, in my opinion, that people have been arguing about whether the leaf points up, or down, without actually realising on what branch they are standing. And it is a very important thing in this risk assessment—if we are to do it properly and responsibly—that we come to realise that this is a tree, that some branches are high, and some branches are low, and that it doesn't matter if the leaf points up or down if you are going to fall 130 feet. Of

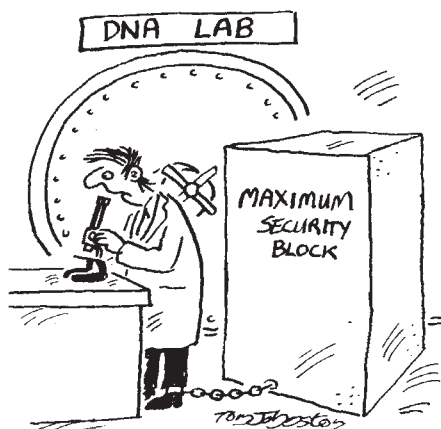
course it matters quite a lot if you are only a couple of inches from the ground because there won't be a leaf, but I'm just trying to say that the business of doing it by example and counter-example—that is, to offer an example scenario that you could in this way create a thing, without having any true estimate of the abundance of these examples and counter-examples—has seemed to me to have be-devilled this field right from the beginning.

And so I had a strong belief that what is necessary in companion with the control of the activities and the categorisation and so on is that it is necessary for all of us—I mean scientists and public participants—to get together and analyse the risks. The existing guidelines are wrong.

Now the guidelines are wrong in an interesting way. I think they are wrong because they have not been scaled with respect to each other. That's the first point. I think they are wrong because—and this I find particularly bad, as a biologist—because they seem to me to perpetrate biological myths—that is myths about the world—which don't exist any more. In large measure they resemble the view of biology which in fact underpins the guidelines in antivivisection: the concept that if you are cold blooded animals you don't feel pain, because the idea is that warm blood and emotion and pain all go together. (You can take frogs and maul them around as much as you like—but not cats and not dogs and not horses. That's an interesting biological classification.)

There is a strong scientific onus that we do *not* enshrine in legislation myths of biology. That seems to me to be an intellectual responsibility that we should share. But I think that there is another responsibility, which is the social one, and that is that we should not be imposing on a subsection of our scientific community and technicians and practitioners controls that appear to them—and objectively—completely extreme compared to what is going on in other fields.

Indeed, if one analyses this, a strong case can be made out that genetic manipulation is actually a method of attenuating dangerous things, and that it is not in itself intrinsically dangerous. In fact it can be argued very strongly that it is a way of containing things, of moving them away from organisms that are their targets, and locking them up in other organisms where they can do nothing. For myself, if there were some national emergency overnight that scientists had to get to work quickly on lassa fever, I would say that the first thing that we should do is clone it in bacteria. Let's clone it in bacteria, let's lock it up, because there it is rendered non-infectious. Then we



can work with lassa fever virus sequences safely in bacteria. (Let me just say that I think lassa fever virus cloning in bacteria is a big no-no experiment, both here and in the United States, and it would take the direst national emergency to make me work with lassa fever virus, and I'd be

very, very careful myself!)

But this is a paradox of the whole field—I put it to you—it sounds awful, but I think I must put it as strongly as that—I would work with the lassa fever virus in *E. coli* any day. I'd consider that to be my best guarantee of safety.

I think you must ponder these things because at first sight they appear totally paradoxical. That is because we have not thought out the hazards directly. I think we have not solved all the problems, and I think there is an enormous amount of work that still remains to be done. 9

New diseases in new niches

By Jonathan King

6 RECOMBINANT DNA certainly represents a major breakthrough in our ability to study the organisation of the genetic material of higher organisms; and I should say that from the research point of view recombinant DNA technology can be employed safely. Although in order to employ it safely you have to assess very carefully what's dangerous about it. But perhaps more relevant here is the new production technology, technology that will be used to manufacture commodities for sale. The transformation from research tools to production technology has proceeded far more rapidly than many scientists envisioned. Within a year or two in the United States Eli Lilly corporation expects to be producing human insulin through the growth of thousands of gallons of *Escherichia coli* containing human DNA sequences spliced into a bacterial plasmid.

Now the deployment of new production technologies has more often than not been associated with the generation of unfortunate side effects on the health and welfare of the human population—most notably those employed at the point of production. I have here a few historical examples. For example the mechanisation of cotton textile manufacture resulted in a drastic increase in damage to the respiratory tract of the operatives (byssinosis or brown lung). Developments in the German chemical industry—such as the synthesis of the aniline dyes which were used to colour the textiles—entailed the production of potent bladder carcinogens—4-amino-biphenyl and β -naphthylamine.

Most of us can be reasonably assured that most of those chemical carcinogens that are already out there through previous mishandling will not reproduce and increase themselves in the environment. The risk is finite. In the case of bacteria we do have to worry that these organisms—or at least the genes that are linked to the plasmids within these bacteria—will move through the ecosystem, transfer for example from the debilitated strains to wild strains of bacteria, and get into strains which perhaps are well-adapted in a particular niche out there. And then we won't be able to clean them

up, for you can't remove, for example, *E. coli* from the ecosystem. It is an intimate part of mammalian life.

Now at this point I wish to clarify and punctuate a very crucial aspect of risk assessment. In trying to assess hazard we must consider what would be the properties, for example, of a wild strain of *E. coli*—even an epidemic strain of *E. coli*—expressing, for example, the human gene for insulin. Now some in the audience will heatedly reply—or they would if I were at home—“but they'll never get into wild strains, they're in debilitated strains, you've got nothing to worry about, you're raising a false spectre”. This is of course putting the cart before the horse. The only way that hybrid DNA will be contained, is if people understand that if it is *not* contained there may be problems.

Thus in assessing the risk of cotton dust, we do not examine the effect of cotton fibres on human skin; we examine the effect of cotton fibres on human lungs. Of course, manufacturers in the industry often say “no that's wrong, because the cotton fibres will never get into the workers' lungs”. But we know that that it is only if people understand acutely what will happen if those fibres *do* get inside the lung, that action is taken. And knowledge in the past has not been sufficient, it's taken much more action than knowledge.

I must ask: where have infectious diseases come from? After all if I'm going to make a feasible case that there is something to worry about in generating a new human disease, I'm behoven to explore the question of the generation of old ones.

The virulent form of *cholera Vibrio* infection, with characteristic rice-water stools, was first reported in the highly densely populated and unsanitary city of Calcutta in 1817. From there it was carried by the British navy to the newly emerging industrial areas in the north-west of England. Manchester in this period was rapidly converting from cottage production of cotton textiles to full-scale factory production. The workers needed to man the mills were either forced off agricultural holdings or one way or another brought into the

city, and essentially forced to live in housing not of their own design. The nature of this housing is quite well documented—miserably crowded, very little light or ventilation, no sanitation, no proper water supply, no means of disposing of waste, thus garbage and excrement polluting the waters used for drinking and washing and food preparation.

Cholera thrived in these industrial districts because these organisms multiply in the intestine, where they elaborate a toxin; and this toxin binds and penetrates the cells of the intestine, and inactivates a protein of the intestinal cells which is needed in protein synthesis. The organism however goes out in the faeces, and if you live in an area with a contaminated water supply and you drink that stuff—boom—you get cholera.

The growth of textile manufacture in the north of England, and also in the midlands, gave rise to many other niches. One of them was damaged lungs from cotton fibres, particularly among operatives of the carding and combing room, where the cotton is taken from the boll to the fibre. These individuals were unusually susceptible to tuberculosis and pneumonia infections, since with the primary barrier of the lung and respiratory tract broken, these organisms move down the respiratory tree and eventually get down to the alveoli of the lung, when you have profound tuberculosis and pneumonia.

Given the conditions that they lived in at home, where there was very very close person-to-person contact, contaminated food, no pasteurisation, etc., there were once again created special conditions for these organisms to

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thrive. Remember also that in this period also children were employed in the mills working 12 to 14 hours a day, malnourished, lacking sunlight, they were hypersusceptible to tuberculosis and related infections. The extraordinary mortality rates among children at that time are due in large part to that.

Thus this is a situation in which technological changes created conditions, both in the factory and at home, in which particular strains of pneumococcus, tuberculosis, and cholera could thrive. Fortunately these conditions do not exist any longer. Over the last 150 years through a kind of alliance between progressive scientists and public health people, and—very importantly—the labour movement, which played a primary role in the fight for decent sanitation and public health, we have reached conditions where water supplies and proper sewers are considered social necessities rather than individual privileges, decent food is available to most people.

I have never thought that the dangers of recombinant DNA research had to do with the generation of a spreading epidemic—some organism that would spread throughout the world. Those conditions don't exist now. It's not the nature of mortality from infectious disease. In fact over the past 30 years, the development and the production of antibiotics has created a possibility where even the rare emergence of some of these organisms can be controlled by antibiotics.

On the other hand, recently we've witnessed the ironic side-effects of the mis-use of the antibiotic technology. The Swann committee dealt with the problem here in the UK. In the United States, though we may have had a Swan committee, it came down on the other side, and we still introduce massive amounts of antibiotics routinely into the feed of chickens, cattle, and hogs, and thus into the general environment.

As a result there is intense selection throughout most industrialised countries for organisms bearing resistance to antibiotics. And this very often is coded by plasmids, the very plasmids that are the parents of those plasmids that are used in the recombinant DNA technology. The other problem of course is hospitals in private practice where antibiotics are tremendously overused, once again creating niches. For example a little pool of water on a sink that's not cleaned up as often as it should be, is a niche where bacteria can thrive only if they contain plasmids conferring resistance to antibiotics.

Now let's switch to the current period of time and ask, in industrialised nations, what kind of problems do

we have from infectious diseases? (And again by 'infectious' I don't mean person-to-person spread—I mean parasitic organisms.) I've just looked briefly at North Carolina and New Jersey in the United States. In North Carolina there are still a great many cotton mills, and byssinosis is still present at a very high rate. (That's because in the United States until very recently brown lung was not recognised as a disease. At least no doctor was willing to testify in court that a cotton worker had lost lung function from that disease.) Also the mechanisation of the cotton industry, which involves grinding up the bracts around the boll into a very fine dust—and it is the bracts that are the primary causative agents of lung disease—increases the problems of the cardroom workers with byssinosis.

So these people occasionally go to the hospital, when they get older, and they are diagnosed as having anticema or some obstructive problem in the bronchi and there is some surgical procedure or some unconventional procedure. What happens then, and so much so that it's a major health problem in the United States (and I believe also in England), is they pick up a hospital infection, a hospital pneumonia, because the primary physical barrier has been breached. A very high percentage of time these infections are due to *E. coli*, a strain that in the old days was never ever thought of as a pathogen. *E. coli* is a laboratory bug, it's the medical students' bacterium, it's something we don't have to worry about.

In Essex county in New Jersey workers still get bladder cancer at ten times the national average. (New Jersey also has the highest concentration of the dye industry.) These people occasionally go to hospitals (if they have health insurance—in the United States that's only a quarter of the population), and maybe they get chemotherapy for their bladder cancer. And they are immunosuppressed. And as a result of this they pick up hospital acquired infections also very often *E. coli*.

Now these infections are not of the spreading epidemic type. It's a different thing; it's a point source; it's more like the bladder carcinogens coming out of the aniline dye industry. There's a place where the organisms are surviving—there's some reservoir—and then somebody comes in who's debilitated or weakened and they pick up the infection.

I don't mean to scare you—I'm now talking about ordinary antibiotic resistant *E. coli* infections—not recombinant DNA—but just to give you a sense of the extent of this I calculate that at present in the United States each year

90,000 individuals suffer surgical wound infections with *E. coli* with 2,700 associated deaths; 40,000 individuals contract *E. coli* pneumonia infections, with 10,000 associated deaths; and 17,000 people contract *E. coli* bloodstream infections with 4,000 associated deaths. Of course the primary infection is urinary tract infection—about 300,000 cases in the United States, primarily in women.

Now these people are weakened—but that's what happens when you go to the hospital, you're in an automobile accident, or maybe a little baby and your immune system hasn't fully developed; we have a right to be weakened, there's nothing wrong with being weakened. In this period of history the people who get bacterial infections are a different population from those who

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got bacterial infections 200 years ago.

I want to emphasise that one of the problems with these infections is that they carry plasmids specifying antibiotic resistance. And so when the person gets infected (1) the person's already weak; (2) if it's a bloodstream infection it's hard to treat; and (3) the plasmids are specifying antibiotic resistance. The organisms don't necessarily spread through the environment but the plasmids certainly spread from strain to strain. Thus you don't have to pick up a new bug—you pick up a new plasmid.

I mentioned child mortality in the 1800s in Manchester. We don't have that problem now; child mortality has a different character, it's not typhus or typhoid or dysentery; but we do have premature babies who are kept alive in conditions where in the old days they would have died. In the United States these premature infants get a very high rate of meningitis infections—and this is meningitis from *E. coli*. It's a nasty infection, 40 to 80% mortality, and the children who survive have neurological and behavioural abnormalities. The strain of bacterium which causes this—*E. coli* with a K1 antigen—is not a particularly virulent strain of *E. coli*, it's in the mothers, it's out there in the normal population; but if it gets into the meninges, into the spinal cord tissue of a premature infant, then you get a very nasty infection. Again it's special conditions.

Now, let's go back to the older view and say what kind of conditions make bacteria nasty with respect to human disease. Well, we have a few cases:

synthesis of a toxin, would be one case, synthesis of a protein increasing the colonising capacity—the ability to stick somewhere—and resistance to antibiotic therapy. The last one I've covered. Cholera, let me remind you, makes a protein that binds to human cells. Diphtheria toxin—the classic case—comes from infection by a phage, not a plasmid, which specifies a protein that's exported outside the cell: that's the thing that makes you sick.

Now I'd like to note two general features of pathogenesis. First, many of these proteins that are associated with the pathogenic character of historically studied bacteria are coded by plasmids; and the transmission from cell to cell of these plasmids is a major medical problem. (It was in the past, and is presently.) Secondly, the feature of many of these toxins is that they have the property of interacting with mammalian cells. You know most proteins in bacteria don't interact with me. They interact with each other. If you want to get a protein that will interact tightly with me, you go to my cells. Go to my kidney cells, and you'll find many many proteins that interact with kidney cells for example, those proteins on the surface of one kidney cell responsible for sticking to the other kidney cell so that I have a kidney.

A number of people in trying to counter the question of the hazards of recombinant DNA research have said human DNA has been getting into bacteria time and time again all through human history. And it has even been proposed explicitly by the very notable American cell biologist Lewis Thomas, though he was making the opposite argument, that some of the diseases I've talked about, like cholera toxin, that the origin of that protein may have been a rare recombinational event, at some time in history, in which a mammalian gene for protein got into bacteria, and that's the way you got a protein that interacts with mammalian cells. So the model here is that when that happens it can be a very unfortunate event.

Now let me move to the question of whether there is really a serious hazard, or how many hairline cracks are there in Sydney Brenner's aircraft engine, in the introduction of mammalian genes into enteric bacteria? I'm sorry I'm forced into the thing that Sydney describes as example and counter-example, but the risk-assessment research hasn't been done—in the United States it has been blocked, and in my estimation it's been blocked because some risk is going to emerge. It's very unfortunate that one's forced into somewhat extreme examples, because the primary data, which might refute some of these, is not available.

We have genes which increase the pathogenicity of the host organism in the niches *already* occupied—that's one possibility; the second is a gene that makes a protein that allows the bug to grow in a place it didn't before—colonise a new place, stick to an organ it didn't before—for example for *E. coli* to colonise the upper respiratory tract, which it doesn't right now; and the third is a more subtle effect, something I've been very concerned with: interfering with the immune response of the host organism.

What about insulin? I don't know what would happen if a newborn infant picked up an *E. coli* meningitis infection and that bacterium was spooing out human insulin; but it seems to me there's every reason to be concerned. And I can't tell you what would happen if the genes for somatostatin, another potent hormone which has been cloned for commercial manufacture, were to be put into a wild strain of *E. coli*; again there's every reason to be concerned. And any endocrinologist not in the pay of the company producing it would have to give pause to the uncontained DNA.

In terms of a new niche, well certainly one of the classes of proteins that people want to study—I'd like to study it myself—is the class of proteins in human cells that allows one cell to stick to another. It's very important in the area of cancer research: how does a cell know when to stop dividing? One kind of DNA that *will* be cloned is the DNA that makes proteins of the cell surface. So people will be isolating bacteria that express in their surface a protein that involves binding to mammalian cells. There's every reason to think that that might, (though it may not), increase the pathogenicity of the wrong strain of bacteria.

Dealing with the immune system—that's a little more complicated. I have submitted a paper on that to the *Journal of Infectious Disease* called "Recombinant DNA and autoimmune disease" which deals with a model of rheumatic fever, where you get a streptococcal infection, a sore throat, that bacteria shows a protein that looks like your heart protein, your body makes antibody against the bacteria, you get over the bacterial infection, and the circulating antibody attacks your own heart protein, and you get rheumatic complications. Well imagine for example an *E. coli* infection with a protein of the synovial membrane of the elbow. You are circulating antibody against the elbow. That is one of the established models of arthritis: antibodies attacking your own joints.

Now, in conclusion, the hazards of introducing an extraordinarily immense pool of genetic information into wild strains of microorganisms are serious;

every effort must be made to see that the genes stay within the debilitated strains; and that these debilitated strains stay within the laboratory. Now this is going to require absolutely the fullest participation of laboratory workers and production workers. And you are not going to be able to do it without strong union participation.

In the area of recombinant DNA technology, the way you know that these organisms are staying in the safe strains, staying in the strains in which they can be handled usefully from a research point of view and perhaps from a production point of view, is to make sure that laboratory workers are protected. If you can ensure that laboratory workers don't pick up these strains whether in their nasopharyngeal passages, or in a cut, or in a urinary tract infection, then you know they are not getting out into the population. The only way we know that can happen is if that sector of the population is fully involved in the safety standards.

Let me close with the fact that I think that just as in the area of occupational carcinogenesis, of black lung, of brown lung, the protection of people from disease involves a tight alliance between essentially progressive scientists and public health people—people who put that as their primary goal—and the trade union movement. That same alliance is the one that's needed with this new technology, and with many of the other new technologies—and more so now than in the past.

Furthermore that has to happen at the international level; because a small sector of the scientific population which is disturbed by this control, which is disturbed by having technicians having a say in the decisions, flies around to international conferences to figure out how to get out of the guidelines, how to weaken the guidelines, how to avoid having trade union participation, and it can be very important that people like ASTMS make contact, for example, with groups in the United States, the oil, chemical, and atomic workers, the French unions, the German unions, the Belgian unions, who represent the same sector of the population, not only to protect themselves but to make sure that in the long run that we're all protected and that the benefits of this new technology—which do exist, and I do believe in them—are realised.

My last point is: don't trust the NIH. Sydney Brenner was right that a better precedent has been established here than has been established in the United States. A fortune is going to be made from the cloning of insulin in bacteria. Four million doses are sold three times a week in the United States. They are not going to sell that insulin cheap; they are going to sell it expen-

sive, because it's human insulin. They are going to *produce* it cheap. That is a very very powerful force behind the scenes, but it penetrates the scientific deliberations. So that in the United States we have scientists who in their public statements say "I'm only interested in the increase of human knowledge", while at the same time they have engaged lawyers to dissociate themselves from NIH funding, and get

private funding, so that they can take out the patents.

Now we don't have a law in the United States that keeps anybody from introducing any gene into an epidemic strain of *E. coli* or *salmonella* or *Shigella*; and there are forces in that direction because of patents. If somebody else has a patent on making insulin in *E. coli* and you recognise that market what do you do? Take two

of the venture capital corporations in the United States, Genentech and Cetus Corporation, both in California; the one has backing from International Nickel and the other from Standard Oil of Indiana. Their front men may be research scientists who say "we are only interested in the expansion of human knowledge", but the background there is very different.

ASTMS plans to radicalise university and health service research

Sheila McKechnie talks to Robert Walgate

THE Association of Scientific, Technical, and Managerial Staffs (ASTMS), which called a meeting on genetic engineering last Friday, has its eye on health and safety in university laboratories. Laboratories in the health service are not left out either. "Freedom of research," says ASTMS health and safety officer Sheila McKechnie, "means whatever anybody wants it to mean at the time. And in the academic context it means that academics don't want to accept either social criteria for their decisions or an input from trades unions."

"If we've got standards of safety in GMAG laboratories it does seem to us that we should use GMAG as a comparison for work with dangerous pathogens; and if that is causing confusion in certain people's minds, then that's unfortunate, but when you talk about laboratory control and labora-

tory safety we are looking to the GMAG model of containment for lots of other areas.

"You've got to remember that prior to 1974 [the date of enactment of the Health and Safety at Work Act] there were no legal standards which applied to the vast number of laboratories in which our members work—which is private research laboratories, public research laboratories, university laboratories, and National Health Service laboratories. Now you might say it's a very weak trade union argument to say that when you get a law you start doing something about it; but in fact it's a pattern of a great deal of trade union activity that when you get a law which protects in a *minimum* way the trade union movement, which hasn't previously seen it as an area for bargaining to be done in, sees a new impetus and sees the law as a minimum and begins to negotiate in that field."

"Laboratories in industry are more hygienic than the university or hospital

laboratories. There's not the kind of scrimping on safety equipment that there is in a lot of hospital laboratories. They are probably more aware of their economic liability if anything goes wrong."

"The problem is the decision-making structure for the allocation of safety money. If unions like ASTMS push for grants from central government to improve laboratories, we would also want some control over how that money was spent. And there is almost total resistance to ASTMS having any say in both the amount and the allocation of that money. The problem in the universities is the anachronistic authority structure within those institutions; heads of departments in universities really are the last bastion of unilateral management prerogative. That is not the way that private companies work any more: they've been forced to recognise trades unions and work with them."

'RECOMBINANT DNA' means different things to different people. Although it is now almost universally associated with the techniques of *in vitro* genetic manipulation, confusion can still arise when the term is loosely applied. (As in some comments on the cause of the recent case of smallpox in Birmingham.) For the products of 'old-fashioned' recombination—the natural reshuffling of genes to produce bacteria, viruses, animals and men with a novel genetic make-up—are also recombinant DNAs.

So far there are a few statutory controls on work with natural recombinants, even when it involves bacteria and viruses that can cause disease in man. But each experiment in which a recombinant organism is produced using the *in vitro* techniques of gene manipulation developed by molecular biologists come under the scrutiny of the Genetic Manipulation Advisory Group whose recommendations about the conditions in which the experiment should be carried out, are legally backed up by the Health and Safety Executive.

This has led to the paradox that experiments arriving at the same end-product can be carried out freely when the end is to be accomplished by natural recombination but come under scrutiny when *in vitro* recombinant techniques are to be used, and emphasises the difficulties in pro-

Recombination: old and new

ducing a logical and consistent set of guidelines for work *in vitro* recombinant DNAs.

Recombinant bacteria can arise naturally in a variety of different ways:

- Certain bacteria directly exchange their chromosomal DNA with members of their own species or with close relatives.
- Infection with particular bacterial viruses which can pick up pieces of bacterial DNA and pass these on to the next bacterium they infect can also produce a recombinant organism.
- Infection with certain bacterial viruses itself can dramatically change the nature of some bacteria. The ability to produce lethal toxins such as botulinum toxin and diphtheria toxin is conferred on the appropriate bacteria by infection with bacterial viruses which become integrated into the bacterial chromosome.
- Plasmids which can be transferred between quite distantly related bacterial species can transmit the genes for antibiotic resistance.
- Viruses can also exchange genetic material between similar or closely related

types. Hybrid trains of influenza virus, herpes and smallpox, for example, all arise naturally from mixed infections or in mixed laboratory cultures of two or more different strains.

So-called '*in vitro* recombinant DNA work' or 'genetic manipulation', specifically deals with work in which DNA from one organism is deliberately extracted, chopped up into manageable fragments with restriction enzymes and the resulting fragments spliced enzymatically into an appropriate plasmid or virus. This can then be introduced into the required recipient cell, whether bacterial, yeast or mammalian cell. The techniques of the molecular biologist can produce organisms that could have arisen naturally, but in addition they can also produce combinations that could not have arisen naturally. However, all *in vitro* recombinant experiments, whatever their outcome, are subject to GMAG controls in the UK and to the NIH guidelines in the USA.

A further twist to the story was provided earlier this year when American molecular biologists showed that in certain conditions, bacteria were quite capable of carrying out the '*in vitro*' techniques themselves, using their own restriction enzymes to cut and splice foreign DNA into their own genetic material.

Eleanor Lawrence

A man driven by proticity

It came as no surprise, and a great delight, to hear of Peter Mitchell's Nobel Prize, in Chemistry, for his work on the energetics of biological systems. "Chemiosmotic theory" said the television-news lady unflinchingly, while her raised eyebrows asked what on earth chemiosmosis might be. A good question, and several million viewers momentarily joined the slimmer ranks of those who, in response to Mitchell's paper in *Nature* on July 8, 1961, had asked much the same. But before the theory, the problem: how do cells derive useful chemical energy (ATP, adenosine triphosphate formed from adenosine diphosphate and phosphate) by oxidising foodstuffs (oxidative phosphorylation) or by absorbing light (photosynthetic phosphorylation)? Or more specifically, how is ATP synthesis coupled to or driven by the electron transfer reactions of oxidative and photosynthetic multienzyme chains?

Earlier theories (Slater, E. C. *Nature*, **172**, 975; 1953) were based on analogies with fermentative modes of ATP synthesis, as occurs in yeasts or muscle anaerobically by a well characterised set of enzyme catalysed reactions and chemical intermediates. All of the necessary enzymes and metabolic intermediates for this sort of ATP synthesis can be reconstituted into an ATP synthesising system in aqueous solution in a test-tube.

These earlier theories, timely as they were, did not provide for the role of membranes (which are the site of the enzymes of oxidative and photosynthetic phosphorylation), and did not provide a necessary link between solute transport across these membranes and some intermediate part of the coupling mechanism. Nor have the predicted chemical intermediates common to both the oxidoreduction reaction and the ATP synthesis (thereby coupling them) been found, despite intensive search.

Theories for a coupling mechanism using energised protons as the coupling intermediate were published by Mitchell (Mitchell, P. *Nature*, **191**, 144; 1961) and also by R. J. P. Williams (Williams, R. J. P. *J. Theoret. Biol.* **1**, 1; 1961). Mitchell's theory, which he called chemiosmotic, consists of four basic postulates concerning the appropriate membranes of bacteria, mitochondria and chloroplasts. The postulates (Mitchell, P. "Chemiosmotic Coupling in Oxidative and Photosynthetic Phosphorylation". Glynn Research Ltd., Bodmin, Cornwall; 1966) state that

- photosynthetic and respiratory oxidoreductions are reversibly proton-translocating
- so is the membrane-bound ATPase

Peter Mitchell Nobel Prize for Chemistry 1978

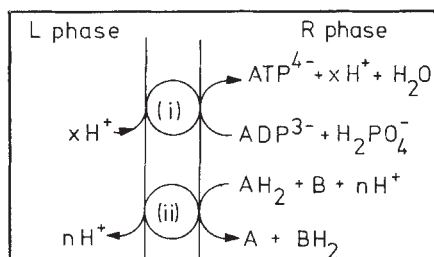
An appraisal by
Peter Garland
of Dundee University



but in the opposite sense across the membrane

- the membrane is impermeable to H^+ , OH^- and cations and anions generally

- nevertheless, the membrane contains specific carrier systems permitting the entry and exit of metabolites in and out of the bacterium, mitochondrion or chloroplast in response to the electrochemical H^+ difference set up across the membrane by the first two mechanisms.



Above: proton-translocating reactions in oxidative phosphorylation, showing a proton-impermeable membrane M that separates aqueous phases L and R from each other. In the membrane are enzyme systems, (i) a proton-translocating ATPase drawn in the thermodynamically unfavourable direction of ATP synthesis, (ii) a proton-translocating oxidoreduction enzyme that oxidises oxidant B, going in the thermodynamically favourable direction. It is postulated that the oxidoreduction, by translocating protons, sets up a difference of electrochemical proton activity—or proton motive force—across the membrane. This proton motive force then drives H^+ from left (L) to right (R) through the ATPase, thereby driving ATP synthesis. Thus the oxidoreduction reaction is coupled to the ATPase reaction by a flow of protons. Similar principles apply for photosynthetic oxidoreductions. A scheme in which protons move from the oxidoreduction enzyme to the ATPase via surface layers of the membrane would not in principle be greatly different, in so far as the high and low energy protons, equivalent to protons on the L and R faces of the membrane, are kept apart by the proton-impermeability of the membrane in what is still essentially a three phase system.

Overall, these postulates linked electron transport, ATP synthesis and hydrolysis, and solute transport, into a single membrane dependent scheme. Conceptually they linked bioenergetics with classical electrophysiology. Because the theory specified aqueous phases bordering the membrane as the phases in which translocated protons appeared, like other transported ions and solutes, it opened the way to experimental test. Herein lay the unique fertility of the theory. It led to a wide new range of experimental approaches directed to the basic postulates themselves and to topics that would have seemed once to be utterly disparate: bacterial chemotaxis, the regulation of intracellular Ca^{2+} , light harvesting by halophilic purple bacteria, biological hydrogen production, to name but a few.

The experimental demonstration of the basic postulates is widely accepted. Less agreed by far are the molecular mechanisms of proton translocation by the ATPase and oxidoreduction enzymes. Nor have localised proton circuits been excluded. Clearly there is much still to be done, and Mitchell's award will most likely mark an acceleration, not a slowing, of research in bioenergetics—a field which must now be all the more respectable. We can also anticipate vigorous attack not only on the underlying molecular mechanisms but also on the theory itself: can it be unequivocally disproven in its present form, need it be modified, is it sufficient? Whatever the outcome, two things at least are sure to remain. Firstly the immense effect of Mitchell's theory in steering bioenergetics away from chemical intermediates into membrane biology. Secondly, the sheer elegance of the experimental tests published in a series of classical papers by Mitchell and his co-worker, Dr Jennifer Moyle.

Historically, Mitchell's interest in the field of biological membranes can be readily traced from at least his postgraduate student days, when his supervisor at Cambridge was J. F. Danielli (it was Danielli who, with H. Davson, had proposed the phospholipid bilayer structure for biological membranes some twenty years before the advent of electron microscopy allowed such structures to be visualised). By 1952 Mitchell was lecturing on membranes and transport to undergraduates at Cambridge; the lectures were memorable for their style, and if we students were confused by them, reassurance came with the thought that the unconfused did not understand the problem. In 1957 Mitchell (Mitchell, P. *Biochem. Soc. Symp.* **16**, 73; 1959) developed his views further in a lecture

to the Biochemical Society, and the idea expressed then that enzymes in membranes could act as (reversible) chemical links between the media on either side of the membrane clearly presaged the full publication of his chemiosmotic theory in 1961, when he was at Edinburgh University.

The following years could not have been easy for him. Leaving Edinburgh in 1963 for health reasons, he set up a private laboratory in Bodmin, Corn-

wall. As a private foundation it was not eligible for support from government research councils. His theory came under severe attack, much of it based on the failure of critics to understand the postulates. In retrospect it might seem that the work of formulating his theory was trivial compared to that involved, under difficult conditions, in testing and explaining the theory to its present stage of recognition.

The word chemiosmosis has been

much argued over, and diversely interpreted. Some say that "osmosis" is wrong. As usual, Popper already has it sewn up, and advises—"Never let yourself be goaded into taking seriously problems about words and their meanings. What must be taken seriously are questions of fact, and assertions about facts: theories and hypotheses; the problems they solve; and the problems they raise". □

news in brief

● **Foreign experts to assess Germany's nuclear plan:** To delay a political storm over the West German federal government's decision to build a nuclear reprocessing plant and storage installation, Premier Ernst Albrecht of the state government of Lower Saxony, where the nuclear facility is to be built, has set up a panel of international critics of nuclear energy to review the plans. This is the first time a European government has invited foreign experts to assess civil nuclear policy. Walter Patterson of the Friends of the Earth, and a member of the panel, told *Nature* that the panel is not just concerned with "the technical nuts and bolts of the project, but is to look into its social and political implications to provide a panoramic overview".

Lower Saxony contains most of West Germany's salt domes, which the nuclear industry and government consider to be the most suitable sites in the country for final storage of high-level radioactive waste. The federal government in Bonn asked Ernst Albrecht to choose one of three recommended domes where the facility should be built. He rejected those three and chose one at Gorleben, which is very close to the border with East Germany. With pressure on one side from Bonn, and on the other from local people and environmental groups hostile to the plan, Albrecht set up the Gorleben International Review in June this year.

The review panel includes ten experts from the US, four from the UK, four from Sweden and two from France. Among them are Professor Karl Morgan, one of the founders of health physics, Paul Sieghart, chairman of the International Commission of Jurists, Dr Frank Barnaby, director of the Stockholm International Peace Research Institute, and Dr Gene Rochlin, who is a member of the Interagency Review Group on nuclear waste management. The panel will submit an interim report probably in February 1979, after which a wide-ranging public hearing will be held. The final report is expected to be submitted by April 1979. □

● **Ultraviolet radiation risk ignored:** Research workers and students in British universities may be risking severe damage to their eyes and skin because safety measures for the use of ultraviolet light in laboratories are being ignored, according to a report by the National Radiological Protection Board (NRPB).

The report states that university staff showed "a general lack of awareness" of the hazards from ultraviolet radiation and of health protection measures. "In general, the standard of engineering controls was inadequate, administrative controls were poor, and although personal protection was available in some cases, there was no warning sign instructing staff to wear it."

Over-exposure to ultraviolet radiation can cause severe skin rashes and can damage the eyes. The main effect on the eyes is photokeratitis, or "arc-eye", which, although not permanent, is extremely painful.

The board investigated 55 installations of ultraviolet equipment in three British universities at the request of their radiation protection officers. Of 24 potentially high risk installations only two had appropriate warning signs, and in only three cases did the staff wear adequate protective equipment such as goggles or gloves.

Very few cases of actual injury have been reported, but as the use of ultraviolet radiation in laboratories increases, says the report, accidents will become more common unless safety measures are taken. Although only three universities were visited, the NRPB regards the attitude to safety it found as probably typical of most research departments. □

● **More funds for reactor safety research:** The West German federal government has decided to increase its expenditure on research into the safety of light water nuclear reactors. This has now become the most rapidly expanding area of nuclear energy research in Germany. DM575 millions (about £160 millions) of government funds will be spent over the four years 1977 to 1980 on expanding and improving the existing reactor safety research programme. This follows the DM318 millions (about £90 millions) spent between 1972 and 1976 on over 600 projects.

The new research programme will concentrate on ways of improving the reliability of reactors, and on what would happen in the event of a breakdown, including an analysis of emergency cooling and melt-down of the core, and the exposure of workers to radiation. □

● **New Fermilab director:** Professor Leon Lederman (right) is to be the next director of Fermilab, the US National Accelerator Laboratory at Batavia, Illinois, which contains the world's highest energy (500 GeV) particle accelerator. However, he will not take acting director, until 1 June 1979 over from Dr Philip Livdahl, the because of his commitments at Columbia University where he has been for more than 25 years. He is also the director of Nevis Laboratories for experimental research in high energy physics.



Professor Lederman, a popular international figure in high energy physics, led the team that discovered the Upsilon particle in 1977, which indicated the existence of one, and possibly two new quarks. In the past he participated in the first observation of the non-conservation of parity in muon decay, and in the demonstration of the existence of two different kinds of neutrinos.

Professor Lederman succeeds Dr Robert Wilson who has been the director of Fermilab since it was founded by the US Atomic Energy Commission in 1967, but he resigned in July this year after a dispute over funds for Fermilab's Energy Doubler project known as "Tevatron", which allow external target experiments of up to 1,000 GeV. □

correspondence

Whither the Natural History Museum?

SIR,—In reply to Dr Halstead's article (26 October, page 683) I would like to say that in my view it is a mistake to suggest that the museum must be either a popular or elitist place. There can be no rigid dichotomy and it is our stated aim to attract an audience representative of the public as a whole. At the same time we wish to present modern science. This means dealing with some difficult concepts, and the communication of these concepts in an accessible but non-trivial way is a serious matter. Dr Halstead will not even identify this as a problem until he is able to distinguish between the communication of information and the simple presentation of facts.

Secondly, it is astonishingly naive for anyone to suppose the existence of such a thing as theory-free observation. Whether we are in a science museum or at a laboratory bench, the theories we already hold play a major part in deciding what observations we make. Without some theories we are lost. It is unreasonable to expect all visitors to a public museum to provide their own conceptual structures.

Lastly, Dr Halstead fails to point out that 15 members of the Museum's scientific departments have been seconded in the last four years, to join 15 scientists already in the Department of Public Services, in the design of new exhibits. A senior member of the Fossil Reptile Section worked virtually full time for nine months on the redisplay of dinosaurs, and was also involved in the writing of a book to accompany this splendid exhibition, which is now taking shape.

Yours faithfully,

R. S. MILES

British Museum (Natural History),
London, UK.

Can India afford to save the Taj Mahal from corrosion

SIR,—It goes without saying that the Taj Mahal is one of the finest examples of the many endeavours the ancient and rich civilisation of India has contributed to the world. The efforts to avoid possible corrosion by the proposed oil refinery (7 September, page 4) must however be analysed in full cognisance of the actual political and economic reality of the country.

Indian bureaucracy is well known for its inefficiency and unchanging social habits in India are well known for maintaining poverty and disease. Any effort to forestall the installation of the refinery at the proposed site has the ominous possibility of entailing a delay of several years before the project gets off the ground again. During this period, a segment of the population would remain unemployed and the earnestly

needed hard currency would have to be spent for oil purchases from abroad retarding not only the goal of self sufficiency in this sector but also all those projects that could be realised with the foreign exchange thus saved.

After spending a considerable sum of money and planning, can a poor nation afford a luxury which even rich nations have to forego, especially since the arguments for the ill effects are meagre indeed? Thus, protests by the Environment Protection Agency were overruled by the US congress during the installation of the Alaska pipeline. And the nature of 'emergency' in a rich US has nowhere near the same dimensions as in India, still dependent upon foreign patronage for development. Among other examples, Egypt did not abstain from the Aswan Dam project just to boast of the original temple sites but keep its people in misery.

The laudable goal of the critics could only be applied if the country were ready for this luxury and could at least provide a minimum for its people. Once the precedent is set, other sites for this and other projects could still have other types of objections and the vicious circle would have no end. It is to be hoped that those who thrive by glorifying the dead would have at least some concern for the living.

M. K. AGARWAL

Paris, France

Trypanocides

SIR,—Your correspondent J. P. Cavill's astonishment (12 October, page 476) at what he considers to be the erroneous statement that Berenil "... is at present the only effective trypanocide left for use in cattle ...", a quotation from a review I wrote in 1976 (*Trop. Dis. Bull.* 73, 531), suggests that he may not be fully aware either of the rest of my review or of the current state of trypanosomiasis chemotherapy in African cattle.

A little more familiarity with the topic, fully documented in my review and elsewhere, would show that as a result of widespread development of resistance to Anttrycide and Ethidium since their introduction over 20 years ago, their use and effectiveness have declined; in some areas, such as Northern Nigeria, they were withdrawn from field use as long ago as 1965 (Mulligan, H. W. "The African Trypanosomiasis" (1970) Ch. 7). Even the more recently structurally-related variants like Prothidium and Samorin have not been fully effective, because of cross-resistance or of damaging dermonecrosis at the injection site, a form of local toxicity common to all active phenanthridinium trypanocides including Ethidium.

In these terms, the sole remaining acceptable drug is Berenil, which, with its freedom from local or systemic toxicity, its high therapeutic ratio, its activity against many resistant strains, and its apparently low potential for the

induction of drug resistance, can properly be described as "... the only effective shot left in the locker for treatment of cattle trypanosomiasis" (*Trop. Dis. Bull.* (1976) 73, 531).

J. P. Cavill maintains that "... "The facts are that several other products are available". For the benefit of the interested reader, and possibly of J. P. Cavill himself, the six drugs other than Berenil which he lists as being available, are in reality only four in number. Trypanidum and Samorin are different trade names for the same compound (originally isometamidium), and Ethidium and Novidium differ only in the nature of the salt ion.

Availability in any case is no guarantee of ultimate effectiveness in African field conditions, where after 20 years of use, Berenil can still number fewer disadvantages than any other drug for the treatment of cattle trypanosomiasis (cf. Finelle, P. (1973) *World Anim. Rev.* (FAO) 7, 1).

Yours faithfully,

J. WILLIAMSON

National Institute for Medical Research,
Mill Hill, UK

Browsers and their browse

SIR,—English-speaking ecologists, especially marine ones, seem to be groping for an appropriate general word to designate the kind of food which grows in swards or pastures. For instance, Caswell (*American Naturalist* 112, 127 (1978)) discusses, in theoretical terms, 'grazing' on 'pastures', whereas Lubchenco (*American Naturalist* 112, 23–29 (1978)) refers to browsing by the snail *Littorina* on the alga *Chondrus* as 'predation' on 'prey'. The latter terms, though well suited to a lion-lamb relationship, seem hardly appropriate for a lamb-grass relationship, though admittedly there are ecological parallels in the two systems. Actually, we already have in English several potentially useful words for this purpose, of which 'browse' (*n.* or *vb.*) or 'graze' (*vb.*) may be the most suitable. With such a term one can discuss the relationships of grazing animals such as parrot-fish or snails to their 'browse'—a carpet of algae, sessile animals, or both—without debasing the meaning of the word 'prey', which typically connotes more violence involved in the process of chasing, catching and eating food.

Note that the terms 'carnivore' and 'herbivore' refer to the feeder and specify the nature of its diet, whereas 'prey' and 'browse' refer to the fed-upon, and respectively indicate whether it has the potential to escape or not. In general, carnivores and their prey have to expend more mechanical energy in chasing, or in trying to evade capture, than do browsers and their browse.

Yours faithfully,

RALPH A. LEWIN

JAMES T. ENRIGHT

La Jolla, California, USA

news and views

New light on the Piltdown hoax?

from L. B. Halstead

THE announcement by Charles Dawson and Arthur Smith Woodward on 19 December 1912 at the Geological Society of London of the discovery at Piltdown, Sussex, of a Palaeolithic human skull and mandible created a sensation. The anthropological establishment in Great Britain was in a state verging on despondency, as a consequence of human skeletal remains, Palaeolithic cave paintings, and human artifacts being discovered in enormous profusion in France and Germany. The revelation of Piltdown man, named *Eoanthropus dawsoni*, went a long way towards raising English anthropological morale. A few voices of dissent were raised but caution was swept aside in the enthusiasm engendered by the very idea that the first man was after all, an Englishman.

Subsequent discoveries, by Dart and Broom of australopithecines from Africa rendered Piltdown man more and more anomalous so that it became something of an anthropological embarrassment. Eventually, when the Piltdown skull was subjected to sophisticated analysis it transpired to be a deliberate hoax. The investigations of J. S. Weiner, K. P. Oakley and W. E. Le Gros Clark (*Bull. Brit. Mus. (Nat. Hist.) Geol.*, 2, 6, 1953; 1955) finally exposed the enormity of the deception. Not only was the Piltdown skull itself fraudulent but the entire mammalian fauna of the gravels had been planted and the human artefacts manufactured.

All the circumstantial evidence pointed to Charles Dawson as the perpetrator and Weiner's book (*The Piltdown Hoax, O.U.P.* 1955) leaves the reader with the unequivocal impression that he was the culprit. Yet there have always been some nagging doubts about apportioning blame to this Sussex solicitor alone.

It was generally felt that there must have been someone with considerable expertise as well as with a known animosity towards Smith Woodward, who must have been ultimately responsible. Various names have been canvassed but, for some curious

reason, suspicion has never been directed to the one man who was acknowledged as a leading anthropologist of the time, who clearly detested Smith Woodward, who had been responsible for exposing Smith Woodward in a description of another hoax and finally who had been almost alone in Britain in giving unequivocal support to Dart's interpretation of *Australopithecus*; a man of whom Smith Woodward had written "he delighted in discussion and controversy, in which his pleasant and occasionally sarcastic humour, his command of information, and the often unexpected turn of his attack, rendered him a doughty antagonist" (*Obit. not. R.S. London* 2, 265; 1938).

Evidence pointing to a new suspect in the Piltdown hoax would perhaps never have come to light, had it not been for the fortuitous circumstance that the man who succeeded to his

"HE [Dawson] of course, was a man who was known to have committed many fakes, and as the bones were found, so to speak, almost on his own back doorstep, he was naturally a strong suspect. There would be many, however, who thought that he must have had a colleague with a much more advanced knowledge of anatomy, palaeontology and palaeolithic man, and who would have had a much more easy access to many of the items found in the fraud.

As I am no longer able to see, to write, or to read, I was determined to make a tape record of my ideas, hence the following notes.

A grudge against one of the principals. Obviously Smith Woodward would suffer and did suffer most. Did they ever try to find out if Smith Woodward had any particular enemies who might do such a thing? No. They did not. And if they had done, they would, I am quite sure, have come face to face with my predecessor at Oxford, namely Professor Sollas. I know, having worked with Sollas for 30 years, that he had a bitter dislike of Smith Woodward which I

University Chair chose to retire to the Isle of Wight, where his neighbour was the amateur vertebrate palaeontologist Richard L. E. Ford. For many years Ford was a friend of the late Professor J. A. Douglas (1884-1978) who held the Chair of Geology at the University of Oxford from 1937 to 1950. Over a decade ago Douglas had spoken to Ford about Piltdown but he had never published the information he had, because he had always felt that he should keep silent out of deference to the family of his predecessor. Nevertheless, Ford persuaded Douglas to put his views on record before he died. The tape was played publicly for the first time at a recent symposium of Comparative Anatomy and Palaeontology at Reading. In the following quotation Professor Douglas suggests a new candidate for the role of Piltdown hoaxer.

think was mutual. I could give many examples but possibly one will suffice. On one occasion Smith Woodward wrote to Sollas and asked if he could send one of his students to work in our laboratory on the ichthyosaur skulls, of which we had a number. Sollas flatly refused. Smith Woodward asked me to intervene and try and persuade him which I did and it was absolutely useless. Nothing to do with Smith Woodward would he ever have in the department. I know that Sollas knew Dawson and had visited him, but on how many occasions I cannot say for I was abroad at the time. Dawson, a Sussex solicitor, would be unlikely to have most of the items which were included in the fake, whereas Sollas had easy access to anything like that through the Museum at Oxford.

In the evidence against one of the suspects, Theilhard de Chardin, I believe mention was made of fragments of mastodon bones. This I think was later dropped. It is a queer coincidence that in late 1910 I sent Sollas the lower jaw of a mastodon and several fragments of bones from Bolivia.

Some of the fragments of the skull

were stained with potassium bichromate and I consider this most significant. I can remember as if it was yesterday a small packet arriving at the museum which Bayzand, the assistant, and I unpacked and found to contain potassium bichromate. We both said "What on earth's the professor ordered this for?" When I mentioned this to Oakley he said "Oh, that might have been used for photography." Now Bayzand was an expert photographer and did all Sollas's photography for him. Hence his surprise at a substance that he knew that Sollas would never have used in the department for that purpose.

Then I remember Sollas borrowing apes' teeth from the Department of Human Anatomy at Oxford, but where would Dawson have got such things except from a colleague? Does it not strike one that Sollas, who was one of the leading anthropologists of the day, is conspicuous by his absence from the picture in Burlington House with Smith Woodward and his colleagues examining the Piltdown skull?

When the skull was proved to be fraudulent it would appear obvious that

Dawson must have had an accomplice. The bones did not originate there but were planted there and it is most unlikely that a Sussex solicitor would have such bones in his possession. They must have been provided from an outside source by someone who had higher scientific attainments than Dawson and it is possible that the thing started as a joke and then got out of hand. I am not suggesting that Dawson knew Sollas and Smith Woodward were bitter enemies, but it may have been put to Dawson that to make a fool of a man of the standing of Sir Arthur Smith Woodward would be very, very difficult and if successful would be, so to speak, the highlight of his many fakes.

Next comes the crucial question: where could they look for a man able to supply the material and capable of doing such a thing for the purpose of belittling Smith Woodward's work. My answer is Sollas, who had already shown that he was capable, having done an almost similar act to exactly the same man in the case of the Sherborne horse's head."

science master to Smith Woodward, who described it at the Geological Society in 1914 (*Q. Jl Geol. Soc.* **70**, 100; 1914).

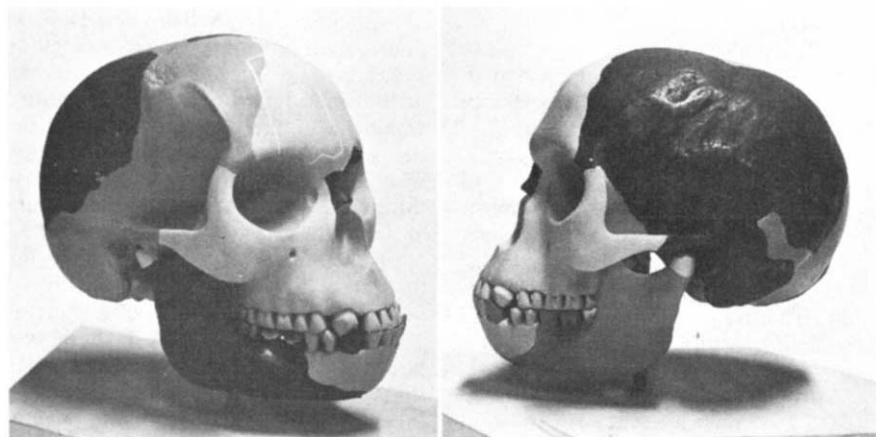
Sollas knew the specimen was a hoax and made no effort to warn Smith Woodward. It was not until 1924 in the third edition of his *Ancient Hunters and their modern representatives* that Sollas announced that it was 'a forgery perpetrated by some schoolboys'. Commenting on this Douglas says "I maintain that a man who could do this once was perfectly capable of doing it a second time".

Two years later Smith Woodward wrote to *Nature* (**117**, 86; 1926) insisting on its genuineness. This brought forth a devastating response from both

Sollas and C. J. Bayzand (*Nature*, **117**, 233; 1926).

Sollas hardly appears in discussions of the Piltdown hoax. Weiner in his book (*op. cit.*) wrote 'the palaeontologist Sollas certainly expressed the prevailing view when he wrote "in *Eoanthropus dawsoni* we seem to have realised a creature which had already attained to human intelligence but had not yet wholly lost its ancestral jaw and fighting teeth . . . It was a combination which had long been previously anticipated as an almost necessary stage in the course of human development.' Sollas's discussion of Piltdown man is worthy of more extensive quotation than this. He compliments Smith Woodward on his reconstruction of the skull, which "he accomplished with great success." He refers to Keith's two restorations which led to Woodward revising his earlier one, and Sollas for good measure illustrates all four versions.

Sollas points out that the skull 'resembles existing men rather than the extinct Mousterians'. 'The brain case, although it presents many archaic characters, is truly human, but this cannot be said of the lower jaw, which is as distinctly simian.' 'The strange creature who thus combined a human brain case with an ape's jaw cannot be included in the genus *Homo*, and a new genus has therefore been created to receive it.' 'Some have regarded such a being as an improbable monster and have suggested that the jaw may not have belonged to the skull, but to a true ape. The chances against this are, however, so overwhelming that the conjecture might be dismissed as unworthy of serious consideration. The suggestion thus summarily dismissed has since received the distinguished support of my friend Prof. Boule and has been strongly advocated by an American naturalist Gerrit S. Miller.' Sollas refers to Pycraft who 'subjected Mr. Miller's arguments to a masterly analysis and ruthlessly tore them to pieces one by one.' This is a telling phrase since Pycraft was the Museum's bird expert and was known to have had less knowledge and understanding of human and ape anatomy than even Smith Woodward. Sollas later draws particular attention to the large bone artefact that Dawson and Smith Woodward found in 1914 and described to the Geological Society (*Q. Jl Geol. Soc.* **71**, 144; 1915.) 'But the Piltdown bone is *sui generis*, no other Palaeolithic age has produced anything like it.' Smith Woodward in his book *The Earliest Englishman* (1948) wrote that they 'were surprised to find that the damaged end had been shaped by man and looked rather like the end of a cricket bat.' What more appropriate tool could there possibly be for the earliest Englishman but a



The reconstructed skull

cricket bat? One of the outstanding characteristics of Smith Woodward was his ability to describe what was before him in exquisite detail but he seemed to have been devoid of a sense of humour, imagination or common sense as is evident from Sollas's note on the Sherborne Horse's Head. Sollas was undoubtedly one of the most important anthropologists of his day. It is not unlikely that Sollas, being acutely aware of Smith Woodward's lack of knowledge in matters anthropological, may have felt that Piltdown would have been an ideal vehicle through which to expose Woodward's claims to virtual omniscience in palaeontology. He clearly must have reckoned without the wildly enthusiastic reception which greeted Piltdown man. The sudden appearance of the fossil cricket bat should, however, have been enough to halt the onward rush of the Gadarene

anthropologists over the precipice. It did not.

When Dart described *Australopithecus* in *Nature* (115, 195; 1925) the Piltdown team of Sir Arthur Keith, Professor G. Elliott Smith, Dr W. L. H. Duckworth and Sir Arthur Smith Woodward roundly attacked his interpretation (*Nature* 115, 234; 1925.) As Dart had stressed here was an ape-like fossil with features of the skull that were essentially human in spite of the smallness of the cranial capacity and also with a human-like jaw. The very antithesis of Piltdown. Next Sollas entered the fray and from his analysis of the skulls of apes and *Australopithecus* confirmed Dart's interpretation concluding 'Australopithecus makes a nearer approach to the Hominidae than any existing anthropoid ape' (*Nature*, 115, 908, 1925).

From the moment of the discovery of *Australopithecus* it must have been evident to Sollas that it would only be a matter of time before Piltdown Man met his final demise. □

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Of mice and men

from Paul Tolstoshev

In recent months, some of the intricacies and mysteries of eukaryotic β -globin gene structure have been unravelled, through the use of recombinant DNA cloning and restriction endonuclease mapping. A successful antenatal diagnosis for a rare form of thalassemia has been made by direct gene analysis of restriction endonuclease generated fragments of DNA from amniotic fibroblasts, and two groups have independently obtained a fairly detailed map of the human β and δ globin gene region.

Since the discovery of an intervening sequence in both mouse and rabbit β -globin genes (Tilghman *et al.*, *Proc. natn. Acad. Sci. U.S.A.* 75, 725; 1978; Jeffreys & Flavell *Cell*, 12, 1097; 1977), it has quickly been established that in the mouse, this intervening sequence, and also a second smaller one, are transcribed into the RNA precursor of β -globin mRNA (Tilghman *et al.*, *Proc. natn. Acad. Sci. U.S.A.* 75, 1309; 1978; Kinniburgh *et al. Cell*, 14, 681; 1978). A second, non-allelic mouse β -globin gene has now been cloned and characterised by Leder's group (Tei-meier *et al. Cell*, 14, 237; 1978). This β -globin gene, found in a 14-kilobase

DNA restriction fragment, also contains an intervening sequence of similar size and location to that in the first cloned β -globin genes, contained in a 7-kilobase fragment. Heteroduplex analysis of the two cloned sequences revealed homology only in the coding sequences and in a few hundred base pairs immediately adjacent to them, in the 7,000 base-pair region which could be compared. In the intervening sequences, homology was only found immediately adjacent to the coding sequences, and at least one half of the intervening sequences did not form a stable duplex. If these two β -globin genes arose by duplication of a common ancestor, then the intervening sequences and the surrounding sequences have clearly diverged much more rapidly than the coding sequences, perhaps, as suggested by the authors, to reduce the probability of further recombination events and effectively to 'fix' the two genes in the genome.

The seemingly general occurrence of intervening sequences in eukaryotic genes, their possible functions and evolutionary origins have been the subject of intriguing speculation in these pages (see, for example, *News & Views* 271, 501; 272, 581; 273, 267; 1978). Comparison of the nucleotide sequences of flanking and intervening homologous regions in the globin genes

(see Miller *et al. Nature* 275, 772, 1978; van den Berg *et al.* this issue of *Nature*, page 37) and certainly in the near future, in α globin genes as well, will be of great interest. Potentially regions of DNA involved in regulating the coordinate expression of the globin genes could be identified.

Human gene mapping

Human β globin genes have also been closely scrutinised by several groups. There are two adult β -like genes, the β and δ genes, and their globin chain products differ by only 10 amino acids. Genetic evidence from the fused δ -N terminal, β -C terminal haemoglobin Lepore has long suggested that the β and δ genes are closely linked. This has now been directly confirmed at the molecular level (see below). Hybridisation data suggests great homology in the nucleotide sequences of these genes; as a consequence, a β -globin specific probe will also detect δ -globin sequences. Mears *et al.* (*Proc. natn. acad. Sci. U.S.A.* 75, 1222; 1978) have used the Southern technique to transfer restriction endonuclease fragments of total human DNA to millipore filters, which were then hybridised to β -globin specific cDNA probes. *EcoRI* digestion produced three β and δ containing fragments. The β globin mRNA sequence is known to contain one *EcoRI* site; presumably the δ globin has one also. Thus, three or four *EcoRI* fragments containing β or δ sequences would be predicted depending on whether there is an additional *EcoRI* site between the two genes. This in fact, seems to be the case, but the fourth fragment hybridises poorly to the cDNA probe and is difficult to detect. When Mears *et al.* similarly analysed DNA from two very rare forms of thalassemia; $\delta\beta$ -homozygous thalassemia, and Hereditary Persistence of Fetal Haemoglobin (HPFH), which are caused by extensive deletions of both δ and β gene coding sequences, two of the three normal β or δ globin-containing *EcoRI* fragments could no longer be found.

Antenatal diagnosis

An obvious application for such analyses is in the antenatal diagnosis of thalassemias, such as $\delta\beta$ -thalassemia, and α -thalassemia, where gene deletions are known to occur. Sufficient DNA can readily be obtained from amniotic fluid fibroblasts, which are easy and safe to collect. This avoids the risks inherent in taking a foetal blood sample to measure globin chain synthesis in the red cells. A molecular hybridisation approach to the antenatal diagnosis of α -thalassemia has already been pioneered by Y. W. Kan and his coworkers and successfully used in several diagnoses. However, this approach requires relatively large

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amounts of DNA and careful controls with DNAs of known α -gene content, and so again restriction endonuclease mapping should prove simpler.

It is testimony to the remarkably rapid pace of progress of this area of research that a successful diagnosis, for $\delta\beta$ -thalassaemia, has already been made by restriction mapping, by Orkin and his coworkers (*New Engl. J. Med.* **229**, 166; 1978). The analysis was performed to confirm the results of a chain synthetic ratio study, since the newer technique is obviously still in its infancy as a clinical tool. Orkin *et al.* were able to show that normal human DNA, digested with the restriction endonuclease *HindIII*, generates three β or δ globin-containing fragments. With DNA from an authentic case of $\delta\beta$ -homozygous thalassaemia, two of these fragments were absent, but in the DNA from amniotic fibroblasts of the fetus diagnosed, these same two bands were present, though at lower intensity than in normal DNA. This obviously suggested $\delta\beta$ -thalassaemia trait (heterozygous), the diagnosis established by chain synthetic ratios, and not $\delta\beta$ -homozygosity. Orkin and coworkers also demonstrated the feasibility of this procedure for diagnosing α -thalassaemia. A single *EcoRI* DNA fragment containing all the α globin coding sequences in normal DNA was shown to be absent from placental DNA from a case of hydrops fetalis—the homozygous and lethal condition of α -thalassaemia where all α globin genes have been deleted.

One potential complication in using restriction endonuclease mapping diagnostically is that any point mutation can potentially eliminate a restriction endonuclease cleavage site, or generate a new one. Thus, missing bands could potentially arise from such a mutation (which could be totally unrelated to globin genes), especially if the globin sequence as a consequence is now in a very large DNA fragment, which is known to transfer very inefficiently from gel to millipore filter, or in a very small DNA fragment, which binds poorly to the filter. While such variations in restriction endonuclease cleavage sites doubtless do exist, this problem should be solved by using a number of different enzymes. These procedures will doubtless become a major diagnostic tool for those thalassaemias caused by gene deletions.

Mapping in detail

But restriction mapping diagnosis may not be limited to disease caused by such major deletions. More detailed mapping with a large array of restriction endonucleases may well reveal much more subtle defects, which may underlie the much more common conditions of β^0 - and β^+ -thalassaemia.

In these disorders it is known that no major deletions are present. As discussed by Nienhuis (*New Engl. J. Med.* **229**, 195; 1978) a restriction endonuclease of appropriate specificity has been found which could potentially enable homozygous sickle cell anaemia to be detected.

A detailed restriction map of the human genomic region containing the β and δ globin genes has recently been independently derived by two groups. The more detailed map comes from a collaborative study by R. Flavell, J. M. Kouter and E. de Boer in Amsterdam, and P. Little and R. Williamson, in London (*Cell* **15**, 25; 1978). Armed with a formidable arsenal of restriction endonucleases, they have been able to construct a remarkably detailed and unambiguous map of this region. Although they had a completely pure human β -globin cDNA probe which had been cloned in a bacterial plasmid, they still faced the problem of distinguishing between fragments containing β globin or δ globin sequences. This problem was elegantly solved in the following way. *EcoRI* digestion of human DNA generates three β or δ globin containing fragments (the fourth is difficult to detect, as mentioned above). They suspected that two of these contained β -globin sequences, since they formed more stable hybrids with the β -globin probe. The human β -globin mRNA sequence predicts an *EcoRI* site at amino acid positions 121–122. A mutant haemoglobin, Hb O-Arab, has an amino acid substitution

at position 121 (glutamic acid to lysine) which eliminates this *EcoRI* cleavage site. Hence, in *EcoRI* digests of DNA from such an individual, a new, large band, equal in size to the sum of the two normal presumptive β -globin containing fragments should appear, and this is precisely what was found, confirming the identity of the β -globin bands. The orientation of the coding sequences was also elegantly determined, by cutting the plasmid containing the β -globin cDNA probe with a mixture of *EcoRI* and *HindIII*. The different sized 3' and 5' sections of the β -globin sequence generated in this way could be used as probes to identify 3' or 5' coding regions in the genomic restriction fragments. The battery of restriction endonucleases, used singly and in combination, enabled the construction of a map, showing that the β - and δ -globin genes are separated by approximately 7,000 base pairs, and that both genes are transcribed from the same DNA strand. The β -globin gene contains an intervening sequence of 800–1,000 base pairs, within the sequence coding for amino acids 101–120. A similar intervening sequence is probably also present in the δ -gene.

A similar analysis of DNA from an individual homozygous for haemoglobin Lepore confirmed that this gene consists of a fusion of part of the δ and part of the β globin gene, with the probable deletion of 8.3 kb.

In the same issue of *Cell* (**15**, 15; 1978) J. G. Mears, F. Ramirez, D. Leibowitz and A. Bank



A hundred years ago

THE following statement with regard to Mr. Edison's recent invention appears in the *Times*:—It appears, from the New York papers, that a company has been started in New York called "The Edison Electric Light Company," with a capital of 300,000 dollars. The object of the company is stated generally to be "the production of heat, light, and power by electricity." The present object, however, is to supply a fund which is to assist Mr. Edison in carrying forward his experiments to a point where he shall give a positive demonstration of the powers of his new inventions. Precisely what these inventions are in all their details of transmission of force and the multiplication of the light derived from electricity, Mr. Edison has not yet told to anybody, fearing that the devices may be patented abroad. The invention, as to the use of electric lights, it is said, will not include the use of carbon

points, as ordinarily known in electric lights, but instead the incandescence of a metal simpler and cheaper in every way. Mr. Edison has determined upon the general features of his light, its manner of production, &c.; but in many minor points connected with the distribution of the light for ordinary domestic and business purposes much work has yet to be done. It was at first supposed that 100,000 dollars would be a sufficient experimental fund, but the larger amount was finally determined upon.

THE French Government proposes to do an act of justice in raising the stipends of professors in science and medicine to the same amount as in the case of law and letters, 15,000 francs. Dr. Simplicie, who writes on the subject in the *Union Médicale*, points out how unequally professors of pure science, as botany and chemistry, are rewarded as compared with, say, clinical professors, who can add enormously to their income by private practice. Dr. Simplicie proposes that a maximum salary should be given to the former, and a minimum to the latter. This is a subject that calls for consideration here as well as in France.

From *Nature* **18**, 31 October, 705; 1878.

present a similar map. They used Lepore DNA restriction fragments to discriminate between β and δ globin containing fragments, and a short 3' specific β -globin cDNA to orient restriction fragments. They have also obtained evidence for intervening sequences of about 1,000 bases in β , δ and Lepore-globin genes.

The next targets for both these groups, and others, are obviously the β^0 and β^+ -thalassemic DNAs, and the γ -globin genes, and the results will be awaited with great interest. $\square$

Deep-sea neutrinos

from David Eichler

THE Deep Underwater Muon and Neutrino Detector (DUMAND) is one of the most ambitious experiments ever conceived. As presently seen, it will consist of thousands of photomultiplier tubes and perhaps some hydrophones distributed over a cubic kilometre of seawater, more than six kilometres below the ocean surface. This is deep enough so that from most angles, neutrinos are the only high-energy particles that can reach the detector. The array will measure the energy and direction of the hadronic showers and muon tracks that are generated by interacting neutrinos. Nature produces neutrinos of extraordinary energies, in cosmic ray interactions with the Earth's atmosphere, for example, and with DUMAND, physicists plan to study interactions at energies much higher than can be attained in man-made accelerators.

Besides its use to study high-energy interactions, DUMAND, by virtue of its excellent angular resolution, will be an excellent neutrino telescope. In September, a group of physicists and astronomers gathered at the Scripps Oceanographic Institute in La Jolla, California, to discuss what sort of astrophysical systems might produce enough high-energy neutrinos to be detected by DUMAND. High-energy neutrinos are produced when high-energy protons bombard ambient material. It has been noted (for example, Eichler *Astrophys. J.* **222**, 1109; 1978; Berezhinsky & Prilutsky *Astrofizika Pis'ma* **3**, 152; 1977) that such high-energy collisions may occur at significant rates in quasars, active galactic nuclei, binary systems and young supernova shells that contain pulsars, and supernovae that go off inside interstellar clouds. In all these systems high-energy particles are produced in a medium of considerable ambient den-

sity and high-energy collisions are inevitable. Thus, there is at least a good chance that such systems could be seen with DUMAND.

Much can be learned about potential high-energy neutrino sources from gamma-ray astronomy, since gamma rays are produced in the same collisions that produce high-energy neutrinos. Gamma-ray astronomer T. Weekes (Harvard/Smithsonian) spoke about the very high energy gamma-ray detections of Cyg X3, widely held to be a pulsar in a binary system, and the active galaxy Cen A. These observations are significant because if these gamma rays are generated in high-energy proton collisions then the associated neutrino emission from these objects would be detectable. C. Fichtel and F. Stecker (both of Goddard Space Flight Center) pointed out that present limits on γ -ray intensities imply that any steep spectrum source that is optically thin to gamma rays cannot be a very strong source of high-energy neutrinos, although it may still be marginally detectable by DUMAND.

The most distant, mysterious processes that generate high-energy neutrinos may be the explosions that occur in the centres of active galaxies and quasars. As noted by J. Scott (University of Maryland) and coworkers, most of the neutrino production that is hypothesised to occur in these explosions may take place during the first few hours, when the ejected material is most dense, and most opaque to photons. Since these explosions, as observed at radio wavelengths, typically last a year or so, DUMAND may serve as an 'eye' that will alert astronomers to new explosions.

In marked contrast to quasars, the closest, most familiar, and—in the eyes of many—most amusing source of high-energy neutrinos may be the Sun. Galactic cosmic rays bombard the Sun's atmosphere in the same way as they do the Earth's, producing high-energy neutrinos. However, they are produced in the Sun's atmosphere much more efficiently. This is because the Sun's atmosphere, being more tenuous, allows most of the relevant high energy mesons to decay into neutrinos, whereas most of them would first be degraded by collisions in the Earth's atmosphere. Thus, the disk of the Sun stands out above the atmospheric background of the Earth in high energy neutrinos. Estimates of the count rate are still uncertain—they depend, for one thing, on the extent to which the Sun shields itself from high energy cosmic rays with strong, large scale, closed magnetic field lines—but they do indicate a strong possibility for seeing the Sun with DUMAND.

There is much uncertainty intrinsic

to astrophysical speculation. Despite the uncertainty in extrapolating high energy physics to energy ranges that have not yet been investigated, one conclusion at least can be drawn from the High Energy Neutrino Workshop. As well as an instrument for doing high energy physics with atmospherically generated neutrinos, DUMAND will be a sufficiently powerful neutrino telescope to study a wide variety of astrophysical systems. Certainly the history of astronomy has been that whenever a new window has been opened, be it in radio, X-ray or gamma-ray astronomy, no matter how radical it seemed at first, there have always been new phenomena seen through it. Astronomers and physicists are hopeful this trend will continue into high energy neutrino astronomy.

Photochemical conversion of solar energy

from Anthony Harriman

THE energy crisis of the early 1970s has stimulated considerable growth in solar energy research. Practical means for collection and storage of sunlight offer a great challenge to the scientist; the areas for possible development are virtually unlimited. Many of the latest advances in the application of photochemistry to solar energy research were reported at a recent meeting in Cambridge.*

The photochemical production of a useful chemical fuel is, in many respects, closely allied to the natural photosynthetic process of higher plants. The natural process reduces CO_2 to carbohydrate whilst oxidising H_2O to O_2 but most laboratory models are concerned solely with production of H_2 . G. Porter (Royal Institution) stressed two important points that are valid for all models. First, the cost of conventional power sources is about \$1/Watt whilst the incident solar energy is roughly 250 Watt m^{-2} . Hence, for a model operating at a 10% conversion efficiency, it is necessary that the total cost of energy output be about \$25/ m^2 . Therefore the model must provide a fuel from a cheap reagent such as water and it must be catalytic with respect to all other reagents. Second, if H_2O is the consumable reagent then its oxidation product must be O_2 . Formation of any other product means that production of the fuel will always be

*2nd International Conference on the Photochemical Conversion and Storage of Solar Energy, Cambridge, 10–12 August, 1978.

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accompanied by unwanted side reactions.

It has been known for a long time that there are many simple metal salts which, in acid solution, give H_2 on ultraviolet irradiation. Recently, it has been demonstrated that this reaction can be sensitised to visible light by using such chromophores as tris(2,2'-bipyridyl)ruthenium (II). Thus, irradiation in the presence of methyl viologen (MV^{2+}) or europium (III) leads to net electron transfer; there is no reaction in the dark.

Normally in fluid solution reverse electron transfer occurs rapidly reforming the ground state reactants; this is a great problem when attempting to derive useful work from a photoredox reaction. However, both A. E. Shilov (USSR Academy of Sciences, Moscow) and N. Sutin (Brookhaven National Laboratory), and their coworkers, have shown that the reverse reaction can be inhibited by the presence of an irreversible reductant such as EDTA. Such three component systems lead to the photosensitised production of a reduced material—in this case MV^+ . Addition of a suitable catalyst, such as Pt or a hydrogenase enzyme, leads to formation of H_2 .

In a slight modification of the above model, both Sutin and J. M. Lehn (University Louis Pasteur, Strasbourg) have developed systems that are believed to involve formation of an intermediate hydride. These reactions may be cleaner since they do not need a catalyst. However, all three component systems reported to date involve consumption of one of the reactants so that they do not fully dissociate water.

In recent years, photoredox reactions have been found to proceed at interfaces provided by monomolecular assemblies, micelles, polyelectrolytes, lipid membranes, and microemulsions. The interface often helps to separate the photoproducts but this depends markedly on the nature of the interface, the reactants, and the products. Thus, H. Inoue (Tokyo Metropolitan University) has found that semireduced anthraquinone can be stabilised by incorporation in micelles, and M. Calvin (Berkeley, California) has shown that a latex surface enhances the rate of electron transfer between singlet excited Rhodamine 101 and an aryl amine quencher. Calvin *et al.* (*Nature* **274**, 507; 1978) have also reported that it is possible to transfer electrons and protons across the walls of a phospholipid vesicle.

A simple method for obtaining some power output from an endergonic

photoredox reaction is to use the redox couple as the basis for a photogalvanic cell if at least one of the products is electroactive. Thus, if two inert electrodes are immersed in the solution, a photopotential can be generated when one of the electrodes is illuminated since the illuminated electrode responds to the photostationary state and the dark electrode responds to the ground state equilibrium. The two best known photogalvanic cells are the thionine/iron (II) system, which has been studied since 1940, and the $bipy_3Ru^{2+}$ /iron (III) system recently proposed by Sutin. Kinetic parameters for both cells were fully described by J. Albery (Imperial College, London) and by Sutin (both papers to be published in *J. Photochem.* January 1979). It should be emphasised that for an efficient photogalvanic effect it is necessary that the electrode shows some selectivity for one of the redox couples. For thionine/iron (II), M. D. Archer (University of Cambridge) and M. I. C. Ferreira (University of Minho Braga, Portugal) have shown that Pt responds selectively to the thionine/leucothionine couple due to a layer of adsorbed dye on the electrode surface. Similarly, G. Calzaferri *et al.* (Bern University) have demonstrated an n-conductive SnO_2 ceramic electrode which appears to be selective for the iron (II)/iron (III) couple.

Rapid progress is being made in the development and understanding of photoelectrochemical cells using semiconductor electrodes in contact with an electrolyte solution. Wide band gap electrodes, such as TiO_2 , although stable, cannot absorb an appreciable portion of the solar spectrum. It is, therefore, of considerable interest to sensitise the electrode to visible light either by doping with metal ions or by coating with a dye layer. C. Stalder *et al.* (University of Geneva) have reported that, whilst most divalent and trivalent cations drastically reduce the efficiency of TiO_2 electrodes, incorporation of Cd^{2+} increases the photoresponse to visible light. However, H. R. Sprunken *et al.* (Kiel University) have warned that the concentration of dopant is extremely critical for optimum effects. The sensitisation of TiO_2 by $bipy_3Ru^{2+}$ has been re-examined by H. Hamnett (Oxford University) whilst H. Tsubomura *et al.* (Osaka University) have studied the dye sensitised ZnO system. There are many problems associated with thickly coated dye layers, notably self-quenching, but small increases in photoresponse are obtainable by this technique.

Small band gap semiconductor electrodes, such as GaP, can give a higher solar conversion but are not sufficiently stable for photoelectrochemical cells. However, Calvin *et al.* have shown that

a protective layer of zinc phthalocyanine helped prevent corrosion of n-ZnO electrodes and may be useful for CdTe and GaAs electrodes.

It is difficult to draw real conclusions from a multifarious meeting but some inferences are outstanding. Thus, formation of H_2 using a three or more component system is now trivial but it does not give the required dissociation of water. The variety of uses found for $bipy_3Ru^{2+}$ is remarkable. Is it really the best compound for each specific use or is it used simply because it is well known? Separation of the redox products, either by an interface or on an electrode surface, seems to provide real benefits and may well represent the most amenable method for photochemical storage of solar energy. And finally, the theory and understanding of semiconductors is now at the stage where rapid advancement in this field is expected in the next few years. This is one area of science where engineers, chemists, and technologists seem able to cooperate freely. □

New surprises from Uranus

from D. M. Hunten

UNTIL a few years ago, Uranus was known chiefly as the first planet to be discovered in modern times, and for its remarkable axial tilt, which puts the axis nearly in the orbital plane. Even the radius, and therefore the mean density, was not accurately established until 1970, when balloon-borne telescope measurements yielded a radius of 25,900 km. This radius was accurately confirmed last year during the stellar occultation that is famous for the discovery of Uranus's rings. The density, 1.21 g cm^{-3} , is far greater than Saturn's 0.704, despite the smaller degree of gravitational compression. It is therefore commonly assumed that Uranus contains a much larger proportion than Jupiter and Saturn of elements heavier than hydrogen and helium. This idea seems to be borne out by the great strength of methane absorptions, but a quantitative assessment has proved difficult, for Uranus's atmosphere is also uncommonly clear. In part, the strength of the bands is due simply to the great depth to which light penetrates.

Microwave radiometry has been found to be an effective way to probe these atmospheres to depths as great as

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a hundred atmospheres. In the hands of a group at the Jet Propulsion Laboratory led by Samuel Gulkis, this tool has been exploited for Jupiter and Saturn. Important results are that the temperature increases inwards at a rate consistent with an adiabatic lapse rate, and that the fraction of ammonia agrees well with the fraction of nitrogen in the solar atmosphere. But Uranus does not seem to fit into this satisfying framework: too much radiation comes out between 1 and 10 cm, and now it is found that the 3-cm flux has increased by 30–40% in the past decade. These results are reported by Gulkis, Janssen and Olsen (*Icarus* **34**, 10; 1978) and by Klein and Turegano (*Astrophys. J. Lett.* **224**, L31; 1978). To set this work in context, it is useful to look back at Jupiter.

This planet emits radio waves by at least three processes. At decametre wavelengths, there are intense bursts, many and perhaps all of which are related to an influence of the moon Io working through Jupiter's magnetic field. Decimetre radiation comes by the synchrotron process from electrons gyrating in the field. Overlapping in wavelength, but extending down into the infrared, is thermal emission from the atmosphere itself. The radio part of the thermal emission is the extreme tail of the Planck distribution, and arises at fairly deep levels because the atmosphere is particularly transparent. As was first shown by Field in 1959, the principal absorption is due to ammonia, in the pressure-broadened wings of the 1.3 cm inversion band. Longer and longer wavelengths can be understood as arising from greater and greater depths, as the absorption coefficient decreases. Crudely speaking, the brightness temperature at each wavelength reflects the atmospheric temperature at the level of unit optical depth (or attenuation of $1/e$). Exactly the same notion is applied to the formation of the dark Fraunhofer lines in stellar spectra.

Given a temperature profile (as a function of pressure) and a profile of ammonia mixing ratio, one can compute the radio spectrum and see how well it matches the observations. This has been done in detail by the JPL group. Obviously there are too many unknowns in the problem for a complete *ab initio* determination, but the temperatures are constrained by other information and by the fact that they must somewhere be as high as the highest observed brightness temperature. The ammonia partial pressure can be assumed to follow the vapour-pressure curve at the lower temperatures, and indeed this assumption is necessary for a good fit. At the warmer temperatures, the mixing ratio

becomes constant, and it is this value that agrees so well with solar composition.

The same procedure works equally well for Saturn, but Uranus shows the curious discrepancies already mentioned. The excess brightnesses were first discussed by Gulkis at a NASA Uranus workshop in September 1974, even though the paper has only recently been published. The intervening years have not made the potential explanations (less ammonia or higher temperatures) any more palatable. The density of Uranus suggests that ammonia should be much more abundant than on Jupiter and Saturn; but more opacity would give lower fluxes, not higher. Uranus has not been shown to have a significant internal heat source, and one might therefore suppose that the temperature does not rise so fast with depth. But again, the discrepancy is the wrong way around. The JPL group suggests that ammonia is underabundant by a factor of at least 100 (NH_3/H_2 , by number, 10^{-6} instead of 1.5×10^{-4}). With this assumption, they can get a good fit, but it flies in the face of everything we thought we knew. It seems most unlikely that nitrogen is absent from Uranus; could it be sequestered in some other form than NH_3 ? Ammonia can combine with water vapour to make NH_4OH , or with hydrogen sulphide to make NH_4SH . The hydroxide has too high a vapour pressure to be a really good scavenger; the hydrosulphide is more promising, if there is enough H_2S available. It would have to be five times more abundant than normal, or NH_3 one-fifth of normal. This is the best explanation we have at present, and it may not be good enough for the deeper levels, where NH_4SH thermally dissociates. No suggestions have been made of ways to sequester nitrogen in the deep interior, and any such mechanism would also have to fit Jupiter and Saturn.

Now comes the news that, in the middle of the anomalous region (2–3.6 cm) the flux has been steadily increasing. A decade ago, Uranus's equatorial plane included the Earth; now we are getting a better and better look at the 'north' pole, and more solar radiation is falling on this hemisphere after its 42 years of autumn and winter. It is tempting to associate the observed trend with a seasonal effect, but no physical mechanism is apparent. The deep atmospheric levels of interest are rather like the bottom of a deep body of water: their seasonal temperature variation must be very small. And again, the sign of the effect is backwards; the spring hemisphere should be colder, not hotter, than the autumn one. Klein and Turegano also discuss

possible nonthermal effects, but conclude that they can be ruled out. Perhaps the two hemispheres simply differ in their ammonia content.

We seem to be left, for now, with no plausible explanation of these related anomalies. It can, however, safely be concluded that Uranus is a very different kind of body from Jupiter and Saturn, and it seems likely that further surprises are in store for us. □

Maternal effects in early development

from Gillian M. Morriss

IN all species of animals and plants which reproduce sexually, the terms male and female are designated in relation to the location of the mobile and the static gamete. The degree of specialisation of the two gametes varies considerably in different species, but in general it is true to say that cytoplasmic inheritance is largely or almost entirely maternal. Maternal influences also extend to post-fertilisation stages of development; this is most obvious in viviparous animals, but even in egg-laying species it is the mother who determines the environment in which the embryos will begin their development. The very diversity of maternal effects which were considered within the scope of a recent symposium* was one of its major strengths, since it both focused attention on broad issues of general principle, and introduced the majority of participants to interesting (sometimes bizarre) phenomena outside their own fields.

In higher plants the distinction between male and female gametes is often blurred, with a significant number of plastids being transmitted though the male (R. A. E. Tilney-Bassett & O. A. L. Abdel-Wahab, University of Wales, Swansea). This is most obvious to the layman in the variegated *Geranium* where the maternal plastids are green, and the paternal white. The *Pelargonium* male does even better, providing all the plastids which survive to supply the next generation.

In the animal kingdom, mothers are unchallenged as major providers of cytoplasm to the zygote. Fathers may even be dispensed with altogether for a few generations, as in the virginoparous form of the *Aphid* (A. D. Lees, Imperial College Field Station). In these insects, freedom from the

*The British Society for Developmental Biology held its 4th symposium, entitled 'Maternal Effects,' on 19–21 September. The proceedings will be published by Cambridge University Press.

necessity to wait for fertilisation enables the oocytes of one generation to begin embryogenesis before the mother is born, so that three generations exist in the form of a single pregnant individual.

Grandmaternal effects can also occur during the course of sexual reproduction, wherever a specific area of germplasm is segregated in the oocyte before fertilisation. This is the case in *Drosophila* as described by R. M. Warn (University of East Anglia, Norwich). The germplasm determinants are located in the pole plasm of the egg; during early stages of cleavage some nuclei move down into this area, and most of the cells which form from the combination of nuclei and pole plasm become germ cells. In the 'grandchildless' mutants the pole plasm is not utilised in this way, so that germ cells fail to form and the adults are sterile. Of the vertebrates commonly used in embryological investigations, it is probable that only anuran amphibians have a real area of germplasm in the oocyte (L. D. Smith & M. A. Williams, Purdue University). Ultraviolet irradiation of the germplasm area of the egg results in the very late arrival of germ cells in the genital ridges, an effect which seems to be mediated through cleavage abnormalities in the germplasm area, and to damage to a (maternal) component of the migratory mechanism (probably mitochondria). Irradiated eggs can be 'rescued' by the injection of cytoplasm from another egg.

A mother can endow her oocytes with an embryolethal component. G. M. Malacinski and J. Spieth (University of Indiana) described a number of maternal effect genes in the Mexican axolotl. In the case of the *o* gene, homozygous embryos can be rescued from their fate of developmental arrest at gastrulation by injection of normal cytoplasm into the egg before the first cleavage division. Maternal effect mutant genes are also known in mammalian (mouse) embryos (A. McLaren, MRC Mammalian Development Unit, London). For instance, the hairpin tail (*T^{hp}*) gene is expressed as polydactyly and spina bifida only when it is inherited maternally; matroclinous and patroclinous matings have shown that this is a cytoplasmic and not a uterine effect.

How much do the cytoplasmic components of maternal origin contribute to post-fertilisation development? The role of maternal mitochondria in chick development has been studied using chloramphenicol, which prevents

synthesis of new mitochondria (F. S. Billett, University of Southampton). Embryogenesis was normal at first, but blood islands failed to form and the neural tube did not close, suggesting that the maternal contribution is sufficient for development to the head-fold stage.

Yolk is another component of the oocyte cytoplasm which contributes to post-fertilisation development in most animal species. Yolk proteins are stored in the oocyte in insoluble form in the yolk platelets; they are derived from the soluble precursor vitellogenin, which is synthesised by the maternal liver and secreted into the bloodstream. J. R. Knowland and B. R. Westley (University of Oxford) described some aspects of vitellogenin synthesis in the frog *Xenopus laevis*. One of the most interesting aspects of their work is that vitellogenin synthesis is under hormonal control, and can be stimulated in the male liver by exogenous oestrogen. The capacity of the liver to respond to oestrogen in this way is gained around the stage of mid-metamorphosis, coinciding with the appearance of oestradiol-binding protein in the liver cells.

In discussion, J. B. Gurdon (MRC Laboratory of Molecular Biology, Cambridge) suggested that in Amphibia some gene repressors or activators might be synthesised in the oocyte, and subsequently segregated into different cells, thus determining them as ancestors of specific cell types. Such morphogenetic determinants almost certainly do not exist in the oocytes of amniote vertebrates (reptiles, birds and mammals), but they have been clearly established in gastropod molluscs (N. H. Verdonk & M. R. Dohmen, Utrecht). The determinants appear to be located in the 'vegetal body', a mass of vesicles filled with electron-dense material, which is segregated into a 'polar lobe' at the first cleavage division. The polar lobe is a small sac-like structure attached to the blastomeres, from which it can be removed without damage. The result of this operation (on *Dentalium*) was the formation of a larva without eyes, tentacles, mantle cavity, or heart. Some of these structures, the heart, for example, are directly lobe-dependent; others, such as eyes and tentacles, depend on a tissue interaction involving the polar lobe.

In mammals, the opportunities for maternal effects are greatly increased as a result of intrauterine development: deleterious environmental effects on the maternal body may have adverse effects on the embryo or fetus, and the maternal genotype acting through the uterine environment may modify development. But several recent studies suggest that mammalian embryos have

a considerable ability to defend themselves against adverse environmental factors. In a demonstration M. H. L. Snow (MRC Mammalian Development Unit) showed that when 7-day pregnant mice are injected with the mitotic inhibitor mitomycin C there is a temporary developmental arrest at the primitive streak stage, followed by resumption of development and the formation of embryos at day 10 with almost perfect form but only 15% of the normal number of cells in each structure; by 13½ days these embryos have achieved weights which are 90% of control values. In my own laboratory we have demonstrated a shorter-term example of morphogenetic regulation: rat embryos were exposed to cytochalasin B *in vitro* just before brain tube closure; the cranial neural folds collapsed, but on later transference of the embryos to control medium, the curvature was regained and neural tube closure effected even though it was by then out of phase with the morphogenesis of other structures.

C. Jones and J. Robinson (University of Oxford) described the fetal response to less than ideal maternal conditions at later developmental stages. Guinea pigs were growth-retarded by artificial reduction of the uterine blood supply; various essential proteins (such as components of the pathway of myelin synthesis, and the liver enzyme pyruvate carboxylase) were first detectable 2-3 days later than in controls, but subsequently reached normal levels. The morphogenetic examples of embryonic regulation described above are similar to these biochemical observations in that in both, adverse environmental conditions delay the synthesis or post-synthetic organisation of proteins which are essential for normal development. The ability of mammalian embryos and fetuses to adapt to such conditions may be an important protection against potentially detrimental maternal effects. □

Errata

In the article 'Nucleon-nucleon interactions' (*News and Views* 275, 589; 1978) Bedford College, London, was inadvertently omitted from the reference to results from the BASQUE group.

THE Conveners of the London Conference reported in the article 'Echinoderm biology' (*News and Views* 275, 268; 1978) were R. E. Emson (King's College, London) & E. P. F. Rose (Bedford College), from whom abstracts of the proceedings are available.

review article

Heavy-ion beam inertial-confinement fusion

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This is a survey article covering the current status of inertial-confinement fusion driven by heavy-ion accelerator systems. Although developed only since 1975, system designs of this type appear to provide a convincing basis for developing commercial power from inertial-confinement fusion.

NUCLEAR FUSION reactions, particularly $d + t \rightarrow \text{He}_4 + n$, can be used as a source of heat for producing electrical power. Among possible reactions, the $d-t$ combination, with an energy release of about 18 MeV, has the least stringent requirements (lowest threshold) for thermonuclear burn. The He_4 secondaries could deposit their energy in the reacting fuel, promoting a self-sustaining burn, while each neutron carries 14 MeV out of the fuel into an absorbing blanket. From this blanket, a heat exchanger can be used to generate steam to drive generating turbines as in conventional power stations.

Generation of useful commercial power from a nuclear fusion reaction requires that small amounts of fuel (for example, deuterium-tritium mixture) be heated to temperatures of the order of 10^8 K for a time long enough to obtain reaction of substantial fraction of the fuel. Magnetically confined fusion devices would contain a plasma with density (n) of the order of 10^{14} ions cm^{-3} for a few seconds of self-sustained burn. Inertial confinement¹⁻³ fusion reactors would ignite thermonuclear burn in a very dense plasma ($n = 10^{24}$ cm^{-3}) on a much smaller time scale (10^{-10} s).

While avoiding large-scale confinement in magnetic fields, an inertial fusion reactor would require beams with an instantaneous power of the order of 10^{14} W to deposit their energy in a target a few millimetres in diameter, with a mass of a few milligrams. The targets could be spherical multi-layered structures with a fusion fuel core, to tailor the compression-ignition sequence for optimum fusion yield.^{4,5}

Technological advances in both beam sources and targets during the last few years have resulted in an increasingly optimistic outlook for achieving commercial fusion power through inertial-confinement fusion. Heavy-ion beam fusion conceptual system designs^{6,7}, based on the mature accelerator technology developed for high-energy physics research, provide convincing arguments that suitable drivers can be built for a commercial electrical power generating fusion system.

In the next section I shall compare the suitability of the various intense-beam technologies for ICF power reactor applications.

Principal beam technologies

There are four principal beam technologies with the potential to reach the power levels required for ignition of thermonuclear burn in a small target^{2,3}. Each has unique characteristics which shape the possibility of its applicability to future ICF power reactors. The four intense-beam technologies are glass lasers, gas lasers, electron-beam accelerators and heavy-ion accelerators.

Glass lasers use neodymium-doped glass discs or rods as the lasing medium. Several laboratories have significant Nd-glass laser installations². The operating wavelength is about 1 μm . This wavelength provides a relatively efficient coupling of the laser light to a dense plasma, near the solid surface of a target pellet which is being bombarded. However, glass thermal stress characteristics prevent rapid pulsing; a period of several hours is required between each shot to cool down the laser. Thus, glass lasers are not suitable for a reactor. Furthermore, their efficiency for transforming electrical power into laser light is very small, of the order of 0.1%. This low efficiency is an undesirable feature for a reactor driver. However, these lasers are suitable for compression physics experiments, and at a sufficiently high power, to produce single intense bursts of neutrons from high-gain targets.

Lasers with a large volume of flowing gas as the lasing medium have been developed for fusion application²⁻⁵. The only existing high power gas laser technology, developed most extensively at Los Alamos, is based on CO_2 . CO_2 laser efficiencies are potentially several per cent, and they can be developed to have an adequate repetition rate. However, CO_2 produces relatively long wavelength (infrared) radiation (10 μm). Coupling to the target plasma of such long wavelength radiation seems more difficult to understand and extrapolate to higher beam powers; more complex physical phenomena may be important, compared to shorter wavelength radiation. For this reason, application of CO_2 lasers to reactors remains an open question. Other gas media are being intensively studied to find short wavelength, high-efficiency gas lasers^{2,3}.

A technology for driving fusion with intense pulsed electron beams is being developed very extensively in the USSR, and on a somewhat smaller scale at Sandia Laboratories in the USA. Pulsed Diodes producing 10^6 A of electrons at energies around 1 MV now are operating. These diodes have high efficiency, and can be pulsed at a reasonable rate. There are severe problems, however, in transporting such high-current beams, and in understanding the energy deposition mechanisms which might be used for high fusion gain targets. Proposed beam-transport methods for a test reactor include the use of wires strung to the pellet from all directions^{8,9}. Although this technology is suitable for producing high thermonuclear yields on a one-shot basis, substantial development would be needed to make it practicable.

Heavy ions can be accelerated to high energies and focused on a small target using conventional accelerator methods, a mature technology developed for high-energy physics research^{6,7,10-14}. Complete conceptual designs for accelerator

systems suitable for fusion drivers are now under intense development, and portions of such systems are being constructed and tested⁷. The use of such a highly developed technology allows a realistic estimate of cost, reliability, and all other performance characteristics in advance of actual construction. Purely classical energy deposition is adequate for high-gain targets⁷. All components of a heavy-ion fusion reactor can thus be analysed without further experimental data. It now seems that heavy-ion drivers should be able reliably to provide the power, repetition rate, beam transport, and focusing characteristics required for an inertial-fusion reactor, with driver costs which would remain a small fraction of the total cost of an electrical power generating station¹⁵. Figure 1 shows four possible system configurations.

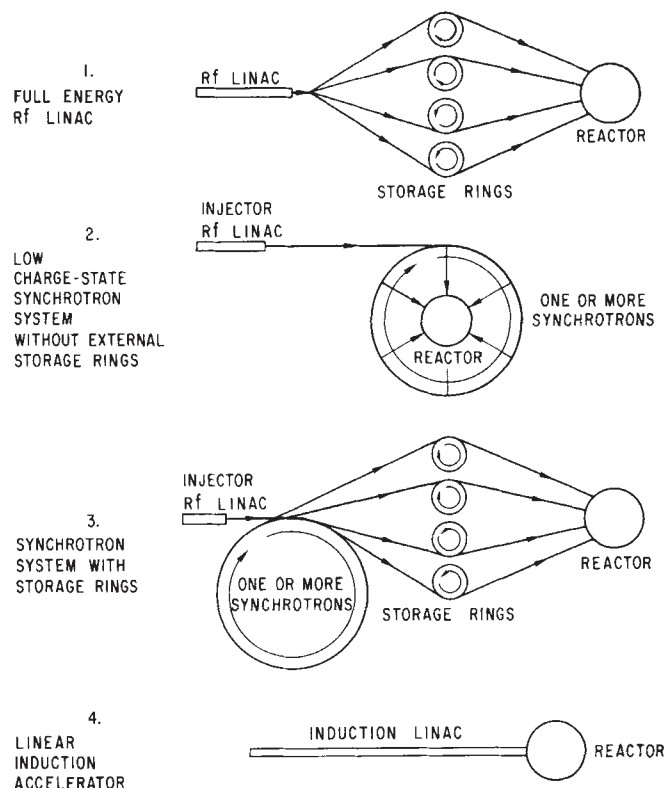
In the remainder of this article I shall discuss design considerations and research required to exploit the ion-beam fusion driver concept.

Design and research considerations

The use of conventional high-energy physics accelerator technology for inertial fusion was first publicly suggested in 1975^{13,14}. The first complete detailed conceptual design for an accelerator driver, HEARTHFIRE (high energy accelerator and reactor for thermonuclear fusion with ion-beams of relativistic energy), was developed by R. L. Martin and me, and presented to an audience of inertial-confinement fusion and accelerator experts in February, 1976; a US patent has subsequently been issued¹².

In July 1976 a two-week summer study on heavy-ion driven fusion, sponsored by the US Energy Research and Development Administration (ERDA), was held at the Claremont Hotel in Oakland, California. Fifty experts concluded there was 'no fatal flaw' in the concept, and that several alternative accelerator schemes were possible⁶. In March 1977, ERDA began preliminary funding of several laboratories for research and development on heavy-ion fusion systems. (I shall sketch the current programmes later.) A second workshop⁷ was held at Brookhaven National Laboratory in October 1977, and a third was held at Argonne in September 1978.

Fig. 1 Accelerator configurations for ion-beam inertial fusion.



Ion sources and pre-accelerators

The source of heavy ions for a fusion-driver accelerator must produce a high current with a very small spread of angles and velocities in the beam. The normalised transverse emittances ϵ_x, ϵ_y of the beam characterise these spreads^{16,17}. It is necessary to obtain normalised emittances appreciably smaller than 0.1π cm mrad, with currents of 10–100 mA, for a source to be useful for a driver based on conventional accelerator designs. (Smaller currents or larger emittances could be used in principle in combination with molecular-dissociation injection¹⁸, but this technique has not been tested.) For some singly charged ions, for example Xe^{+1} , these requirements are being met by sources now under construction⁷.

The species of ion chosen depends on the physical and chemical properties of the element, which govern the technology involved in constructing a high-current source. The noble gases are suitable for a conventional (plasma) source, and Hg is another candidate. High-current Cs sources based on surface ionisation are also available. With such metal ions, however, there are some additional accelerator complications; deposition on the high-voltage accelerating electrodes must be prevented, for example by heating the electrodes. $A > 100$ is desirable, but Rn is unpleasantly radioactive; a convenient choice is Xe.

Higher charge states can be obtained through stripping singly charged ions to a higher charge state in gas or foils at a few MeV kinetic energy¹⁹. A substantial loss of usable ions takes place at stripping, since not all of the ions emerge in the correct charge state. However, for the charge-states and energies of interest, the electrical current (in the correct state) does not decrease, and this is sufficient to allow adequate beams in the accelerator.

The initial ion acceleration from the source to around 1 MeV requires a voltage-multiplying d.c. accelerator such as a Cockcroft-Walton or Dynamitron. Devices capable of accelerating the required ion currents are under development and construction in several laboratories⁷. To minimise the complexity and cost of the following low-velocity RF linac accelerator stages, a high pre-accelerator voltage is desirable. A modified Dynamitron is used in the Argonne program to produce 1.5 MV of pre-acceleration voltage; at Berkeley and Brookhaven, existing facilities provide up to 750 kV.

Acceleration to full energy

The kinetic energy for heavy ions suitable for inertial-confinement fusion lies in the range 3–30 GeV. These energies can be achieved with two conventional accelerator types developed for high-energy physics research, the RF linear accelerator (linac) and the synchrotron. Other methods have also been suggested, including linear induction accelerators (previously used for generating high-current electron pulses) and collective-effect ion accelerators⁶. The two conventional methods require storage rings for accumulation of the required ion currents before discharge onto the pellet can occur. The induction accelerator and collective accelerators might directly accelerate pulses of several kA of instantaneous ion current; however, their technology is not as mature as that of the conventional methods, and it is more difficult to estimate costs and reliability as a consequence.⁷ I shall describe in detail only conceptual designs based on RF linac and synchrotron systems.

Linacs are capable of rapid loading of the storage ring array, requiring only a few milliseconds, in principle.

Synchrotron system designs can be chosen which are less expensive than linac systems of comparable energy, but only if the allowed accumulation time in the storage rings can be made long enough, for example 1 s or longer. The limitations on storage time are discussed in the next section.

Since high currents are more difficult to achieve than high kinetic energies, accelerator systems will typically use the highest kinetic energy allowed by the requirement of efficient energy deposition in the pellet, to achieve the necessary power levels. This maximum energy is set largely by restrictions on the range of the ion, which increases roughly as $(E/A)^2$. For a

targetted energy of 1–10 MJ, typical of predicted requirements for high gain targets^{6,7}, typical maximum energies are 20–40 GeV for $A \geq 100$, corresponding to a range of about 1 mm in a heavy material.

Existing heavy-ion accelerators have been surveyed in ref. 19. All high energy ion machines constructed to date are for the prime purpose of basic nuclear reaction studies, in which the required currents are a small fraction of a millampere. High-mass ions have been accelerated; the Unilac, for example, will accelerate uranium¹⁹, but only in a highly stripped charge state. The vacuum requirements and source current requirements for ion-beam fusion preclude the use of any existing accelerator for experimentation on thermonuclear burn driven by heavy ions.

Storage rings and storage time limitations

The storage of a circulating current of charged particles is limited in quantity by the defocusing effects of their mutual electric repulsions; this limitation is called the 'space charge limit'^{16,17}. For ions of mass number A and charge q (in units of the electronic charge e), with a circulating beam of normalised transverse emittance $\varepsilon_n (= \varepsilon_x = \varepsilon_y)$ in milliradian-centimetre units, the maximum number of ions in each ring is given approximately^{6,16} by:

$$N_{SCL} = 1 \times 10^{13} \frac{A \varepsilon_n}{q^2} \beta \gamma B_f$$

where B_f is the bunching factor (fraction of ring circumference populated by beam) and β, γ are the relativistic kinematic quantities, v/c and $(1 - v^2/c^2)^{-1/2}$.

From this equation, we can see that: (1) low values of q/A are necessary for high energy storage; (2) the total ion energy stored per ring W increases as the square of the kinetic energy E , since $W = E \cdot N_{SCL}$, and N_{SCL} grows linearly with E (in a nonrelativistic approximation).

The values of ε_n are limited to values of order unity by the requirements for focusing the extracted beams onto a small target (a few mm in diameter) situated several metres from the last magnetic lens. For Xe with energies of 20 GeV, $B_f = 1/2$, and $\varepsilon_n = 3$ (typical values), we find the maximum energy stored per ring is:

$$W(\text{MJ}) \sim 4/q^2$$

Charge states of 1–2 would thus allow only one storage ring to be used. However, overall systems considerations (including the accelerator) favour a higher q and several storage rings, for the lowest cost¹⁵.

The storage lifetime is limited principally by losses from ion collisions of two types: ion-background gas and ion-ion collisions. Whenever either collision changes the charge state of the ion, that ion changes its orbit and is lost from the circulating beam.

The background gas collision rate can be reduced to a few percent per second by using a sufficiently high vacuum, for example a pressure of 10^{-11} torr. Such vacua are currently routine at the Intersecting Storage Ring facility at CERN, which stores 2 MJ of circulating 30 GeV proton beams for high-energy physics experiments.

The ion-ion charge-changing collisions are potentially a more troublesome problem, as their rate depends on intrinsic beam parameters and not background parameters. An estimate of this lifetime can be obtained if we assume a spatially uniform beam profile²⁰:

$$\tau = 4\pi R^{5/2} \varepsilon^{1/2} / (N \nu_0^{3/2} v \sigma_{CC})$$

where σ_{CC} is the ion-ion charge-changing cross-section, averaged over the relative collision velocities of the ions in the beam; R is the ring radius; ε is the beam emittance; N is the number of particles in the beam; ν_0 is the tune of the betatron oscillations; and v is the average ion velocity.

The typical collision energy is set, by transverse (betatron) beam particle oscillations, at around one KeV per nucleon. At this energy, for heavy ions, theoretical estimates are quite

difficult, and experiments on appropriate cross-sections are nonexistent at present⁷. Preliminary theoretical estimates of (Y. K. Kim, personal communication) for such energies suggest that σ_{CC} may be as small as 10^{-16} cm^2 for Cs^{+1} and 10^{-17} cm^2 for Xe^{+8} or Cs^{+9} . Lower charge states of Xe could have much larger cross-sections, by as much as two orders of magnitude. Resolution of this issue is likely to have important consequences for the cost of synchrotron-based ion-beam fusion systems. Linac-based systems, although probably more expensive, are capable in principle of filling storage rings fast enough to avoid prohibitive losses from ion-ion collisions, and also relax the vacuum requirements.

Bunching, transport, and beam focusing

The total stored energy of the circulating ion beams must be delivered on the target in a few ns, to produce efficient pellet burn^{21–25}. The circulation time of ions in the rings, however, is typically of order 1,000 ns. Longitudinal compression⁷, and/or subdivision of the circulating beam into many smaller segments which are finally simultaneously extracted and targetted¹² is necessary to match these time intervals.

Momentary longitudinal compression of circulating bunches in the storage ring far beyond the quiescent space-charge limit is possible for a few revolutions. This technique has been demonstrated by Maschke⁷ in a preliminary way using 200 MeV protons in the AGS at BNL. Theories of this compression using RF bunching in the rings, with idealised beam shapes, have been given⁷. Combining fast compression with simultaneous extraction from a few ports around the storage ring allows the total circulating ion beam energy to be delivered in a few nanoseconds to the target, using a few beamlines.

The current ANL conceptual designs¹⁵ utilise one or two beamlines per ring, with several rings. Pulse time shaping, advantageous for high gain targets, can be achieved by a variation in the bunching factor and/or the number of beamlines from different individual storage rings.

The instantaneous beam current in each transport line to the target is several KA (electrical). Space-charge forces are large. Power transport in these beamlines is limited by the strength of magnetic fields which can be utilised in the focusing magnets. Analytic expressions for power limits, based on homogeneous beams, have been published^{6,7}. Further investigations including stability considerations have been summarised in the BNL workshop⁷. Typically, about 10 TW can be transported in each beam-line for Xe^{+8} at 20 GeV, and about 100 TW with Hg^{+2} at 30 GeV, using state-of-the-art superconducting magnets. The power limit also depends on the transverse emittance of the beam. These numbers are representative of a system which uses $\varepsilon_n = 3 \text{ mr cm}$.

The momentum spread in the beam is an important parameter determining feasibility of the final focusing lenses⁷. The focal length of conventional magnetic lenses will be different for differing particle energies; this is called chromatic aberration, by analogy with light optics. Without substantial artificial compensation of this aberration, ion beams useful for pellet fusion targets need a final momentum spread $(\Delta p/p)_T$ of much less than 0.5% to insure the bulk of the beam strikes a target of order 1 mm in radius¹⁵.

Longitudinal pulse compression (as described earlier) increases the longitudinal momentum spread in the ion beam by the compression factor, typically of the order of 100. Thus, to avoid excessive $(\Delta p/p)_T$, a very stringent requirement is necessary on the $(\Delta p/p)$ values in the main accelerator. When rapid-cycling synchrotrons are used, this limits the bunching factor to values which are small compared to typical high-energy research machines. Linac momentum spreads (as injectors, as well as full-energy accelerators) are limited by properties of the initial (low- β) sections. Thus, the entire accelerator system must be properly matched to the target requirements.

Chromatic compensation in the final transport and focusing lenses has been proposed⁷, to allow a larger momentum spread,

and thus a less costly accelerator system. However, no specific design has yet been proposed which is suitable for heavy-ion fusion applications.

Beam propagation in the reaction chamber

After the ion beams enter the reaction chamber they propagate without further external focusing to the target. Space-charge forces could defocus the beam, if sufficiently strong. Although it is conceivable that low charge-state ions (for example, Hg^{+1}) could be used to minimise defocusing, this would necessitate a high vacuum target chamber, to avoid stripping. A high vacuum is undesirable for a repetitive reactor system, since wall damage suppression is easier with a buffer gas fill or liquid lithium protection. Studies have also shown possible two-stream instability problems⁶ with a low pressure gas (tenuous plasma) background.

A background gas pressure of order 1 torr in the reactor vessel offers several advantages: (1) The beam will be charge-neutralised (and predominantly current-neutralised as well) from background electrons (ionised by the head of the beam pulse), thus avoiding defocusing from space charge. (2) Conductivity of the plasma is high enough to avoid development of the hose instability²⁶ over the time and space scales of interest. (3) Collisional damping in the background plasma suppresses growth of two-stream instabilities. (4) Filamentation instabilities do not grow fast enough to be troublesome (with 20 GeV Xe^{+8} beams) (D. Tidman, personal communication). (5) Transport of non-neutron energy from the thermonuclear reaction to the reactor wall can be slowed down to a time-scale of a few ms through fireball formation. Excessive shock overpressures on the reactor wall are not expected for 1-torr gas pressures.

The last consideration is quite important for a reactor, to avoid excessive wall damage by the X-ray burst which would arrive at the wall within a nanosecond after thermonuclear burn in the target. Such reactor considerations are receiving close scrutiny in laser-driven reactor designs, which are very close to heavy-ion reactor designs.

Energy deposition in targets

The kinetic energy of the ions must be transformed into thermal energy in an outer portion of the target, to provide the driving force for compression of the thermonuclear fuel. The slowing-down of individual ions in cold (that is, non-ionised) material is well described by classical theories based on Coulomb collisions with electrons in the material. The same theory is expected to apply in a homogeneous plasma; the stopping power decreases somewhat with increasing temperature. However, for target plasma temperatures below 100 eV, the order of magnitude of the range remains the same as for cold material. Current designs for heavy-ion driven inertial fusion targets^{6,7,23,27} are based on such classical theory. Note the electrons in the target are heated by the ion beams, and the target ions are in turn heated by the hot electrons. The expected equipartition time is less than one nanosecond, based on classical plasma phenomena.²¹

It is possible that additional nonclassical energy deposition mechanisms may be active when sufficiently intense ion beams interact with a hot, inhomogeneous plasma region in the target. Such phenomena have been experimentally observed in the collision of intense relativistic electron beams with targets²⁸⁻³⁰. Their theoretical explanation is not yet definitely established, and may be different under various experimental condition. If present for ion beams, they could further increase the target coupling and lower the cost of accelerator driver systems for a given specific deposited energy in the target.

Target structure

The general principles of fuel compression and ignition for heavy-ion beam driven targets are similar in many respects to laser-driven targets. The principal difference is in the primary

energy deposition mechanism, which is assumed classical in the ion-beam case, but involves collective nonlinear phenomena in laser energy deposition.

Theoretical discussion of the simpler laser targets has been presented by Brueckner and Jorna.²¹ Subsequent publications have described investigations of targets with spherical shells and voids^{23,31-37} which can decrease power and time-shaping requirements and increase gain, compared to homogeneous targets. Symmetry requirements for such targets are discussed in ref. 22.

The first target investigations published appropriate for ion-beam fusion were the single-shell models of Clauser²⁷ in 1975; they required about 400 kJ for scientific breakeven. Multiple-shell ion-beam targets were discussed by Lindl and Bangerter²⁴ which required much less beam energy (50–100 kJ) for breakeven. Further refinements, described by Bangerter and Meeker at the 1977 Cornell Conference²³, have resulted in an example of an unclassified ion-beam pellet design with predicted gains of 88, with an energy input of 1.28 MJ. Such targets may be quite suitable for some power reactor designs, driven by high-efficiency linacs, with repetition rates of 10–20 Hz.

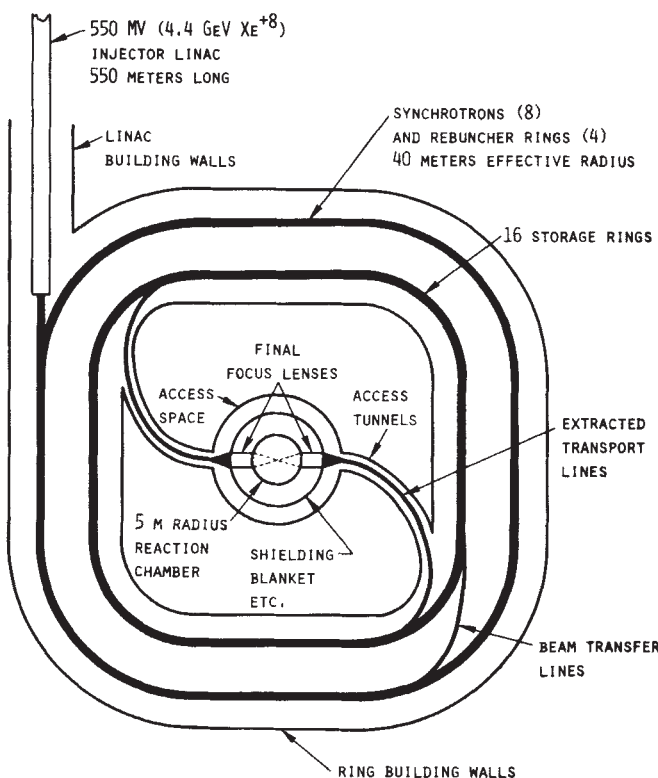
Predicted gains of 500–1,000 for some ion-beam targets designed at LLL were reported at the 1977 BNL workshop⁷. Such targets could allow commercial reactors to be driven by synchrotron systems with a few per cent efficiencies and pellet repetition rates of one per second. The structure of some laser targets of such high theoretical gains have been described by Lindl at the 1978 San Diego Conference³⁸.

If anomalous energy deposition mechanisms operate with intense heavy-ion beams, some of the target design concepts proposed³⁹ for relativistic electron beams might be applied to allow utilisation of higher energy ions, which have lower cost in storage rings for a given total stored beam energy.

Reactor concepts

Inertial-confinement reactor design concepts are very similar whatever the driver technology. Differences arise principally in the allowed gas fill of the reactor chamber. Three of the principal

Fig. 2 Argonne Xe^{+8} synchrotron reference design.



reactor-vessel engineering concepts are: (1) dry wall with gas-filled chamber, (2) wetted wall with liquid lithium coating, and (3) thick liquid lithium first wall.

A comprehensive discussion of a laser-driven fusion power generating station was given by Booth *et al.*⁵ in 1976. More recently, a laser-fusion reactor study based on a graphite chamber wall, with LiO₂ powder as the tritium breeding medium, has been completed at the University of Wisconsin⁴⁰. The engineering for such a reactor appears to be feasible, aside from the laser driver. As a consequence, a heavy-ion fusion reactor seems quite reasonable from an engineering viewpoint, provided that pellet gains of the order of 100 are demonstrated. The principal engineering uncertainty at present seems to be the design of an automated pellet manufacturing facility, which will depend on the structure required in the pellets.

The economics of power generation depend on the cost of a driver system with beam energies of 1 MJ or greater. If such a high repetition rate, long-lived driver can be produced for a cost of \$300 M–\$400 M, then the cost of the nuclear components of a 1,000 MWe fusion power plant should be less than the other components (heat exchanger, turbines, generators, and so on.) Such a generating station would then be economically competitive with nuclear fission stations. The fuel costs are estimated to be small.

Programmes and prospects

Three multipurpose accelerator laboratories in the USA—Argonne, Brookhaven, and Berkeley—are now funded by the US Department of Energy (DOE) for research on heavy ion fusion drivers. Target computations are currently supported at the Livermore Laboratory. Several other small supporting efforts, including beam transport and ionic charge-changing cross-section calculations, are also funded by DOE. The total level of support for fiscal year 1978 is approximately \$4.5 M, with Argonne and Berkeley each receiving about \$1.5 M, and the others somewhat less.

Each of the accelerator labs is developing low energy intense heavy-ion accelerators which might serve as prototype for the initial stages of an inertial fusion driver accelerator. Their experimental programs are summarised in the 1977 Brookhaven workshop. Berkeley has emphasised development of linear induction accelerator technology, while Argonne and Brookhaven have research programs centred on conventional accelerators.

Conceptual designs for a prototype reactor driver are being prepared by each of the three laboratories. After adequate technical discussion on these concepts, the labs are expected to

submit detailed proposals to DOE for construction of a heavy-ion demonstration experimental facility (HIDE). This accelerator facility will be constructed with DOE funding and is expected to cost between \$60 M and \$100 M. The purposes of HIDE are: (1) demonstration of the accelerator technology required for a power reactor, and (2) demonstration of ion-beam energy coupling to a target sufficient to establish the physics necessary for high-gain target designs.

The energy and power levels appropriate to accomplish these objectives are not definitely established at this time, but an energy of at least 100 KJ seems necessary.

Current conceptual designs for heavy-ion inertial fusion systems, based on mature accelerator technology and a modest amount of required development, strongly support arguments that a fusion power reactor could be built on the basis of present technology.

As an example of a 1 MJ (100 TW peak power) driver we describe briefly the synchrotron system currently proposed for detailed study by Argonne¹⁵. The configuration is shown in Fig. 2.

This system uses pulses of Xe⁺⁸ accelerated to 20 GeV, with 8 parallel rapid-cycling synchrotrons, 40 m in radius, operating at 60 Hz. (The ion source produces Xe⁺, which is stripped to Xe⁸⁺ at 11 MeV.) The injector linac accelerates 30 mA of Xe⁸⁺ to 4.4 GeV, and delivers this to all 8 synchrotrons. The pulses from the synchrotrons are accumulated for 1 s in 16 storage rings, 30 m in radius. Final fast compression by a factor of 50 is utilised. Extraction is accomplished into a total of 24 beamlines leading to the targets, in a reaction chamber 5 m in radius. The overall repetition rate is nominally one pellet s⁻¹.

The estimated cost for such a system is about \$250 M, which means it is a strong candidate for an economical power reactor. Other designs (for example, using full-energy RF linac accelerators) may offer technical advantages and higher repetition rates at some additional costs, but still well within the economic range of interest for reactors.

The development of power through heavy-ion driven inertial fusion thus seems eminently feasible, unless pellet classification difficulties² impede commercialisation. This is not likely to be a long-range problem, however, since discussions of unclassified targets with predicted adequate gain have already been published²³, and experiments will check these predictions.

Much material in this article was obtained from the Argonne Accelerator Research Facilities heavy-ion fusion group at Argonne, especially R. L. Martin, R. J. Burke, M. Foss, T. Khoe and S. Fenster. Y. K. Kim communicated unpublished data. This work was supported by the US Department of Energy.

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articles

Ge/Si and Ga/Al variations along the Reykjanes Ridge and Iceland

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Ge/Si and Ga/Al variations emphasise the distinct chemical nature of the mantle present beneath Iceland as compared with the Reykjanes Ridge. Generation of basalts at greater depth beneath Iceland than the ridge is suggested.

THE probable cause of the 400-km long rare-earth concentration gradient found in basalts erupted along the Reykjanes Ridge and Iceland¹ has stimulated heated debates^{2,3}. The subsequent findings that radiogenic strontium and lead contents also decrease significantly along the same profile^{4,5} helped, confining the probable cause of these gradients to mantle-derived heterogeneities rather than their being primarily due to variable extents of: partial melting¹, degrees of equilibration⁶⁻⁸, phlogopite breakdown⁹, fractional crystallisation^{2,10} and/or seawater contamination¹¹.

To cast further light on the phenomenon and to try to resolve the origin of such mantle heterogeneities, we report here on variations of Ge/Si and Ga/Al ratios along the same Iceland-Reykjanes sampling profile (Fig. 1). Ge/Si and Ga/Al constitute two geochemically coherent pairs which are not significantly fractionated during crustal rock evolution.¹² The coherence of the two pairs reflect the similarity of their atomic properties, such as their outer electronic configuration, valence state, ionic radius and ionisation potential.¹³ In a recent study of a wide range of Hawaiian lava types covering the three major tholeiitic, alkalic and nepheline-melilitic lava series, we have shown that Ge/Si and Ga/Al change by only 20% and 62%, respectively¹⁴. Chemical variations in these rocks have generally been attributed to a wide range of fractional crystallisation and partial melting conditions¹⁵⁻²¹, which suggested that as both Ga/Al and Ge/Si ratio are rather insensitive to magmatic processes occurring at relatively shallow depth, the two pairs, particularly Ge/Si, might be useful mantle sources and also depth indicators¹⁴.

Results

Ge, Si, Ga and Al contents and Ge/Si, Ga/Al and Ge/Ga ratios of the Iceland-Reykjanes Ridge tholeiite sampling profile are summarised in Table 1, and their variations along the ridge are shown in Fig. 2. Ge and Ga contents were obtained by neutron activation analysis²², and Si and Al by conventional wet chemistry²³. The Al content along the profile remains constant, averaging 7.6 wt% Al $\pm$ 4% (1 σ). Si remains also constant along the Reykjanes Ridge with a mean of 23.35 $\pm$ 1% (1 σ), but drops significantly over Iceland with a mean of 22.77 $\pm$ 1% (1 σ). On the other hand, Ga, Ge, Ga/Ge as well as Ga/Al and Ge/Si

show two statistically distinct levels north and south of approximately 62°15'N. Only a relatively narrow transition zone occurs between the two chemically distinct ridge segments. Note that the latter profiles are similar to those of radiogenic Sr (ref. 4), Pb (ref. 5) and La/Sm (ref. 1), though the transition zone appears wider for the Pb and La/Sm profiles (Fig. 2). As ⁸⁷Sr/⁸⁶Sr and ²⁰⁶Pb, ²⁰⁷Pb and ²⁰⁸Pb relative to ²⁰⁴Pb variations are generally accepted to reflect mantle heterogeneities and mixing beneath the region^{4,5}, by analogy, it would seem logical to attribute the two distinct Ge, Ga, Ge/Ga, Ge/Si and Ga/Al populations to the same cause. On the other hand, the lower silica observed on Iceland compared with the ridge could be due to other possible factors such as: (1) fractional crystallisation of mineral(s) richer

Fig. 1 Location of basalts studied. Dredged samples were obtained with RV Trident of the University of Rhode Island; fms, fathoms.

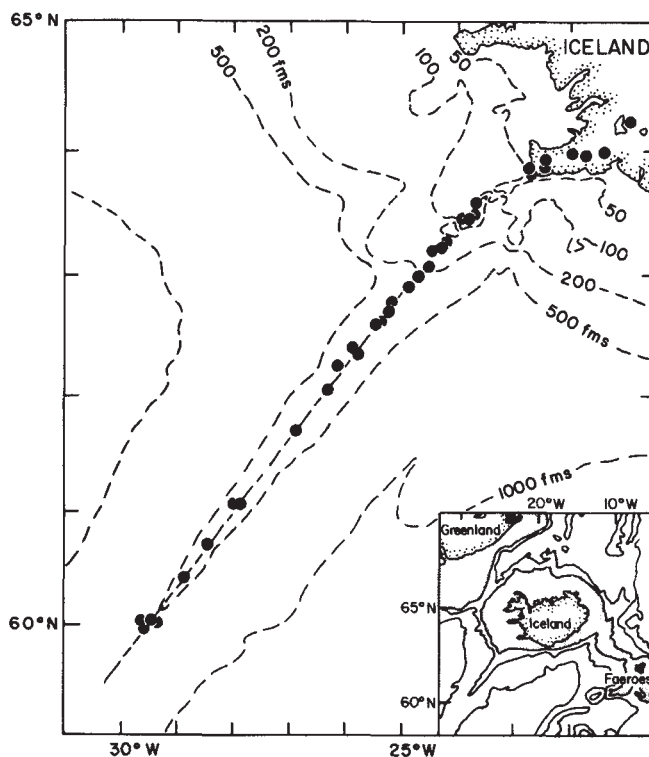


Table 1 Ga and Ge contents of basalts in p.p.m. and Al and Si contents in wt%

	Distance*	Al	Ga	(Ga/Al)×10 ⁴	Si	Ge	(Ge/Si)×10 ⁶
Iceland							
IC-36	-59	7.89	21.2	2.69	22.21	1.40	6.3
IC-43	-24	7.84	21.7	2.77	22.60	1.50	6.6
IC-62	-23	7.46	20.1	2.69	23.00	1.60	7.0
IC-47	-22	8.02	21.9	2.73	22.56	1.47	6.5
IC-58	-14	7.29	21.5	2.95	22.95	1.41	6.1
HS-529	-7	7.22	21.7	3.01	22.95	22.58	98.4†
HS-524	-6	7.96	20.4	2.57	23.00	1.96	8.5†
HS-525	-6	7.69	23.6	3.07	22.85	2.58	11.3†
Reykjanes Ridge							
15D-18A	33	7.32	21.0	2.87	23.40	1.35	5.8
Lynch 18	39	8.42	21.3	2.53	23.51	1.38	5.9
19D-5A	49	7.42	22.2	2.99	23.23	1.60	6.9
18D-1	49	7.10	20.2	2.85	23.30	1.41	6.1
16D-4	49	7.26	20.5	2.82	23.46	1.48	6.3
11D-2	78	7.09	20.6	2.91	23.39	1.41	6.0
12D-40A	86	8.03	21.0	2.61	23.31	1.34	5.8
14D-5C	91	7.08	22.8	3.22	23.52	1.56	6.6
7D-5A	107	7.39	20.0	2.71	23.45	1.51	6.4
6D-9	118	7.55	19.1	2.53	23.26	1.57	6.8
5D-4	130	7.68	21.8	2.84	22.92	1.37	6.0
3D-5A # B	147	7.64	22.1	2.89	23.25	1.50	6.5
35D-3A	160	7.44	20.0	2.69	23.28	1.41	7.0
2D-1,2	172	7.41	22.4	3.02	23.38	1.41	6.0
1D-2	176	7.28	20.8	2.86	23.34	1.60	6.9
22D-1	228	7.45	17.3	2.32	23.62	1.80	7.6
23D-1	210	7.46	17.2	2.31	23.18	1.73	7.5
34D-6C	221	7.60	20.1	2.65	23.30	1.52	6.5
24D-6C	248	7.32	16.4	2.24	23.59	1.70	7.2
27D-11A	298	7.62	18.5	2.43	22.97	1.67	7.3
29D-5A	390	7.64	17.0	2.22	23.44	1.70	7.3
30D-10A	390	7.76	17.8	2.29	23.39	1.86	8.0
31D-9C	440	7.69	17.9	2.33	23.56	1.92	8.2
33D-6A&B	482	8.14	16.6	2.04	23.15	1.71	7.4
D-22-1	543	7.94	18.3	2.31	23.29	1.65	7.1
D-19-1 # 11	544	7.86	18.5	2.35	23.45	1.70	7.3
D-18-2	545	7.56	17.2	2.28	23.44	1.64	7.0
D-38-2	547	7.59	16.9	2.23	23.46	1.76	7.5
Robert Victor Mine Eclogite‡							
Whole rock		—	13.2	1.59	—	1.39	6.2
Garnet		—	13.0	1.09	—	1.60	8.4
Omphacite		—	13.6	2.89	—	1.14	4.4
Rock Standards§							
BCR-1 (3)¶		—	24.0±0.8			1.40±0.08	
JB-1 (7)			18.1±0.6			1.33±0.08	

* Distance in km along Reykjanes Ridge axis, positive south-west with zero chosen at south-west end of Reykjanes Peninsula.

† Samples were taken in an area of intense hydrothermal activity. They are also anomalously high in La and Cl.

‡ Al and Si contents for calculating the Ga/Al and Ge/Si ratios were taken from Philpotts *et al.*²⁷

§ Taken from ref. 14. || One standard deviation. ¶ number of replicate analyses.

in SiO₂ than the primary melts, either at low or high pressure; (2) larger degrees of partial melting²⁴; (3) partial melting at higher pressure beneath Iceland²⁵; as well as, of course, (4) mantle heterogeneities in SiO₂. Although the FeO*/FeO*+MgO of the Iceland and Reykjanes Ridge basalts overlap, the SiO₂ content is consistently lower, and Ga/Ge higher, over Iceland than along the Reykjanes Ridge for equal FeO*/FeO*+MgO ratios (Fig. 3). The presence of basalts with SiO₂ lower than 49%, with basalts having normal 49–51% SiO₂ content, are also commonly found along other isotopically anomalous segments of the Mid-Atlantic Ridge, such as over the Azores and Jan Mayen platforms, 45°N and 35°N regions²³. Note also that these anomalous ridge segments are not uniformly enriched in LILE (large ion lithophile elements) and radiogenic isotopes relative to each other and to normal ridge segments²³. Although on the basis of these observations, we favour production of the low SiO₂ Iceland basalts in conditions of higher pressure and degrees of melting than the Reykjanes Ridge, we cannot rule out that small mantle heterogeneities in SiO₂, and some fractional crystallisation may have also intervened.

Comparison with Hawaiian Islands

More convincing arguments for estimating the relative importance of these processes can be found by comparing Ge/Si and Ga/Al variation in the Iceland–Reykjanes Ridge basalts with those from the Hawaiian islands previously reported¹⁴. Lavas from the Hawaiian Islands cover a much wider range of composition (nephelinitic, alkalic and tholeiitic series), and partial melting and pressure conditions of formation (a range of 0.5–2%, 8–15% and 20–30% degrees of partial melting, and pressure from 20 to 1 kbar, for the generation of primary melts from these three series²⁶, listed in the same order). Large extent of fractional crystallisation at shallow depth have also been suggested within the Hawaiian alkali series^{14,19}. Ge positively correlates with Si and Ga with Al, whereas Ge/Si and Ga/Al changed only by a factor of 1.2 and 1.6 over the entire range of the Hawaiian lava compositions (Fig. 3) (ref. 14). The constancy of Ge/Si in this previous study showed that: (1) during partial melting and shallow depth fractional crystallisation, the Ge bulk crystal/melt partition coefficient *D*, is similar to that of Si, and

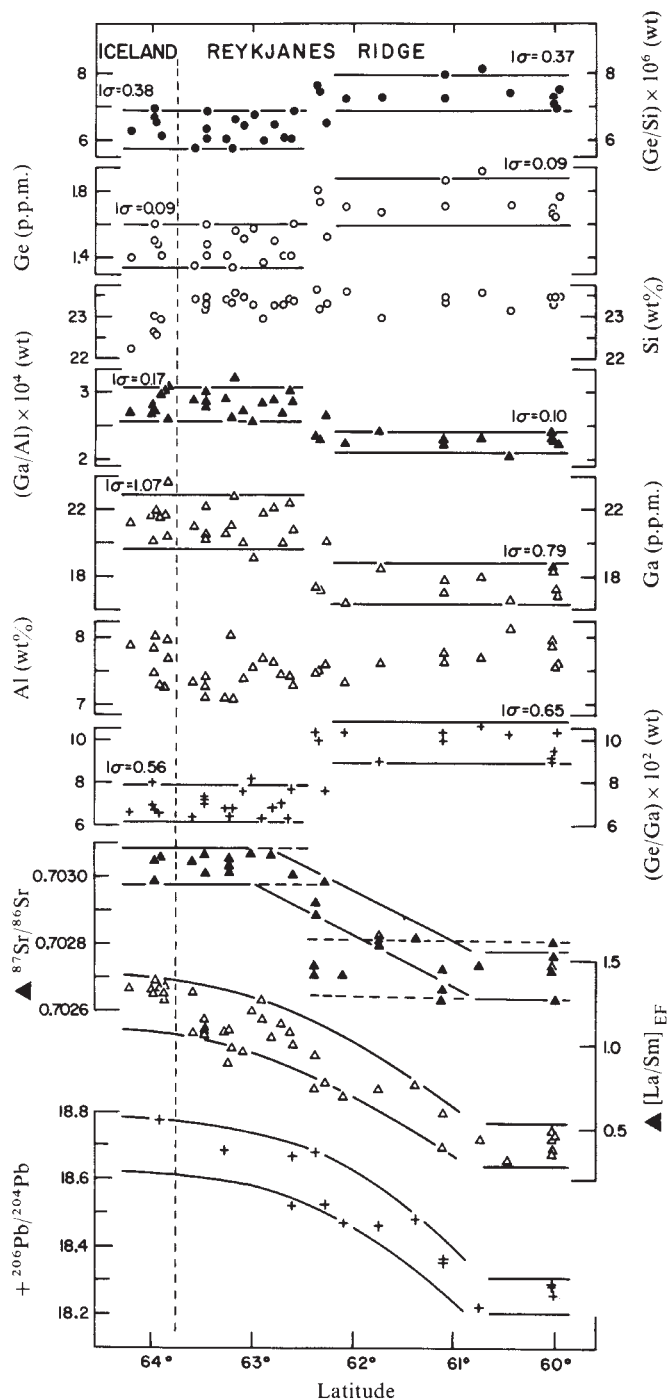
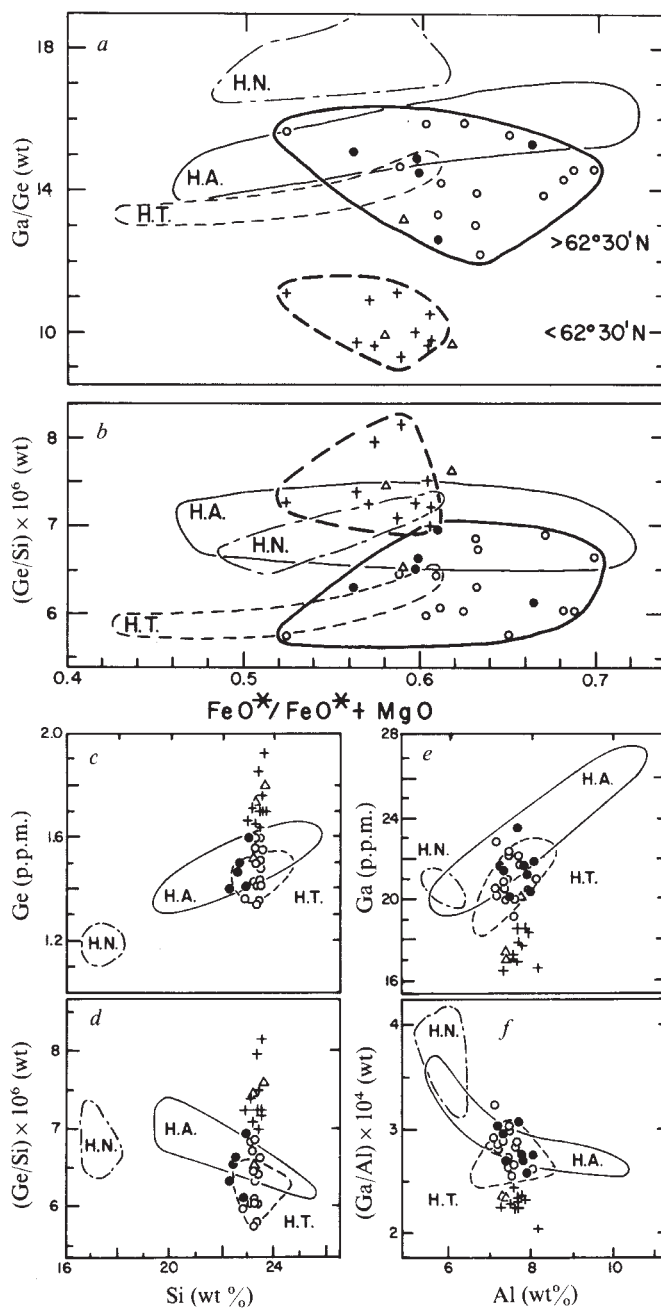


Fig. 2 Ge/Si, Ge, Si, Ga/Al, Ga, Al and Ge/Ga variations along the Iceland-Reykjanes Ridge profile are compared with [La/Sm]_{EF} (ref. 1), ⁸⁷Sr/⁸⁶Sr (ref. 4), and ²⁰⁶Pb/²⁰⁴Pb (ref. 5) variations along the same profile. Note the different width of the transition zones. Envelopes represent ± 1.5 times the standard deviation from the means of the two populations shown, north and south of 62°20'N.

both are close to unity (Table 2) (ref. 14); (2) although D values both for Ge and Si are changing with increasing pressure, and/or decreasing partial melting, their ratio must remain close to unity to within $\pm 10\%$ (Table 2). As a result, we¹⁴ concluded that basalts showing any pronounced variation in Ge/Si would probably have to be attributed to mantle source heterogeneities in Si and/or Ge. Figure 3 shows that the Ge and Ge/Si against Si trend for the Reykjanes Ridge-Iceland is in marked contrast with that for Hawaii. Ge and Ge/Si in the former increase by a

factor of 1.43 and 1.42, respectively, for a change of only a factor of 1.24 for Si. Compared to the three Hawaiian lava series, the Iceland-Reykjanes Ridge tholeiitic basalts cover a much narrower range of SiO₂ content and extent of major element fractionation, but Ge and Ge/Si variations are much greater. The Ge/Si of the tholeiites from Hawaii and Iceland are similar,

Fig. 3 Comparison of variations of Ge/Si and Ga/Ge relative to FeO*/FeO*+MgO for the Iceland-Reykjanes Ridge basalts and Hawaiian lava series (*a* and *b*); Ge and Ge/Si relative to Si (*c* and *d*), and Ga and Ga/Al relative to Al (*e* and *f*) of the Iceland-Reykjanes Ridge and Hawaiian lavas¹⁴. Note, the lower SiO₂ contents of Iceland basalts compared to the Reykjanes Ridge basalts located either north or south of transition zone (*a* and *b*); and the similar Ge/Si and Ga/Al ratios of the Hawaiian and Iceland tholeiitic basalts (*d* and *f*). Fields of HT (dashed line), Ha (thin line) and HN (dotted-dashed line) indicate the Hawaiian lavas of the tholeiitic, alkalic and nephelinitic series, respectively¹⁴. Thick dashed line and crosses indicate the Reykjanes basalts located south of 62°N; ●, Iceland; ○, Reykjanes Ridge basalts north of 62°20'N; △, transition zone between 62°20'N and 62°30'N.



whereas the relatively unfractionated tholeiites from the Reykjanes Ridge south of 62°15'N have significantly higher Ge/Si compared to both Iceland and Hawaiian tholeiites (as well as most Hawaiian alkali lavas and some nepheline-melilitite basalts, see Fig. 3). Finally, Ga/G_e in the three Hawaiian series positively correlate with FeO*/Fe)*+MgO, whereas the converse seems to be true for both the Iceland and the Reykjanes Ridge basalts (Fig. 3). On the basis of these observations we conclude that Ge/Si and Ge/Ga variation along the profile cannot be reasonably attributed simply to different degree of melting and/or extent of fractional crystallisation. We suggest that the mantle beneath Iceland and the ridge north of 62°30'N is significantly depleted in Ge compared to the LILE-depleted asthenosphere source of ridge basalts south of 62°15'N. On the other hand, the relatively low Si content of basalts over Iceland, compared to the Reykjanes Ridge, probably reflect generation of basalt at higher pressure (and/or by greater extent of partial melting). Both inferences are consistent with the plume hypothesis previously discussed^{1,23}, but do not prove its existence.

Table 2 Crystal/melt partition coefficients*

	Low pressure fractional crystallisation† (olivine-gabbro type)	High pressure fractional crystallisation‡ (eclogite type)	
	Effective D^*	K_{garnet}	$K_{\text{clinopyroxene}}$
Ge	0.917	2.80	1.85
Si	0.964	0.81	1.08
Ga	0.750	0.39	0.41
Al	0.213	1.45	0.46
La	0.047	0.01	0.06
Sm	0.283	0.22	0.19

* K_j is for concentration ratio in crystal j relative to the melt. D is the effective partition coefficient for a given process, and is defined as: $D = \sum x_j K_j$; where x_j is the weight fraction of mineral j entering or leaving the melt.

† Estimation based on an effective partition coefficient D^* for the Hawaiian alkali series (from alkali olivine basalt to hawaiite)¹⁴. D for Ge and Ga and La are averaged from Table 3b and Si and Al from Table 3a in ref. 14. The D value for Sm was obtained from variation of Sm relative to La for this series, and using equation (3) in ref. 14.

‡ Crystal/melt partition coefficient for Si and Al were estimated assuming a melt composition of 48% and 15% respectively, and garnet and clinopyroxene compositions from this eclogite reported by Philpotts *et al.*²⁷

Similar arguments can be developed for the Ga/Al variations. However, it is evident in Table 2 (and ref. 14) that Ga and Al bulk partition coefficients effective during partial melting in the upper mantle, and lava ascent, are significantly smaller than unity. Contrary to Ge, Ga is enriched in the Iceland mantle compared with the LIL depleted asthenosphere, and behaves similarly to LIL elements. Furthermore, the greater scatter of Ga and Ga/Al compared with Al as shown in Fig. 3, suggests a lesser degree of geochemical coherence between Ga/Al pair compared with the Ge/Si pair.

Fractional crystallisation models

O'Hara's hypotheses suggesting increasing crystal fractionation towards Iceland, either at shallow depth², or by a combination of high to low pressure regimes¹⁰ (proposed to account for the rare earth variations observed along the Iceland-Reykjanes Ridge profile¹), are now evaluated on the basis of Ge/Si and Ga/Al data. Judging from the fractionation trends of the Hawaiian alkali series, both Ge/Si and Ga/Al should decrease with increasing fractionational crystallisation at shallow depth (such as increasing SiO₂ and Al₂O₃ or FeO/MgO or Larsen index¹⁴). The evidence suggests that only Ge/Si decreases towards Iceland, and Ga/Al does the opposite. Thus O'Hara's low pressure fractionation model is not supported on this basis.

O'Hara's model involving fractional crystallisation from high to intermediate pressure (eclogite) followed by low pressure (olivine gabbro) fractionation, progressively increasing in intensity towards Iceland, is illustrated quantitatively in Fig. 4. Crystal/melt partition coefficient data of Table 2 and the

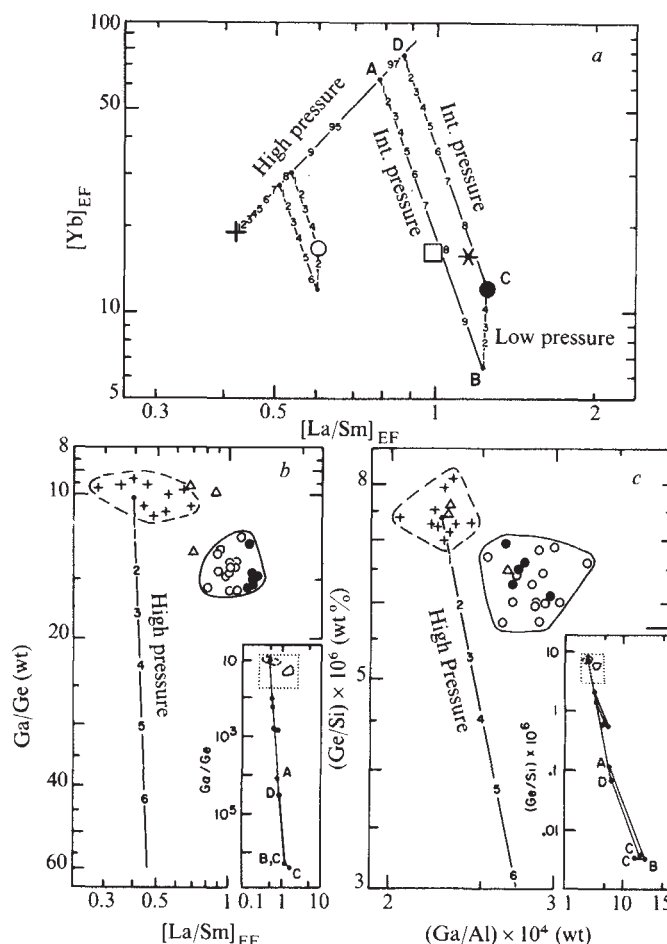


Fig. 4 *a*, Variation of Yb with La/Sm enrichment factors relative to chondrites ($[La/Sm]_{EF}$) in basalts from the Reykjanes Ridge and Iceland, averaged out by segments: +, south of 61°N (arbitrarily taken as original melt); ○, 61–62°N; □, 62–63°N; *, 63–64°N; ●, Iceland (data from ref. 1). Theoretical fractional crystallisation paths followed by residual melts caused by high pressure eclogite (40 kbar, 0.86 cpx; 0.14 garnet) fractionation (for example, line +—A or D), intermediate pressure eclogite (~20 kbar, 0.57 cpx; 0.43 garnet) fractionation (for example, lines A—B or D—C), and low pressure (<10 kbar, olivine gabbro, 0.17 ol.; 0.33 cpx; 0.50 plag) fractionation (for example, line B—C). Numbers 0, 1, 2, 3, 4, 5, 6, 7, 8, 9, 95 and 97 refer respectively to 10, 20, 30, 40, 50, 60, 70, 80, 90, 95 and 97 wt % crystallisation of the mineral assemblages indicated above. Crystal/melt partition coefficients set I in Table 2 of ref. 36, were used, as well as the Doerner Hoskins³⁷ fractional crystallisation law; *b*, Comparison of observed Ga/Ge with $[La/Sm]_{EF}$ along the Reykjanes Ridge and Iceland (data points) with calculated, high, intermediate and low fractional crystallisation paths (lines), corresponding to path +—A—B—C in (*a*) (which links basalts south of 61°N to basalts from Iceland). Note that the Ga/Ge ratio would have to change by more than 6 orders of magnitude to satisfy such a path. The mismatch between such a path and the actual Ga/Ge against $[La/Sm]_{EF}$ plot along the ridge is clear (see insert). Crystal/melt partition coefficients from Table 2 were used for Ga and Ge. *c*, As (*b*) but for Ge/Si against Ga/Al. Note again the mismatch, and the fact that the Ge/Si ratio would have to change by more than three orders of magnitude (see insert). Fields in *a*—*c* are: +, Reykjanes basalts population south of 62°20'N; ○, north of 62°30'N; △, between 62°20'N and 62°30'N; ●, Iceland basalts. Dashed field are basalts south of 62°20'N; solid field are basalts north of 62°30'N.

Doerner Hoskins trace element fractional crystallisation law were used to derive possible extraction paths. For example, path +A-B-C (Fig. 4) assumes that if ridge basalts south of 60°N are primary, Iceland basalts would represent only a 3.9×10^{-4} final residual liquid fraction after extraction of: 98.7% of an eclogite mixture composed of a 0.86 clinopyroxene and 0.14 garnet at high pressure followed by; 93.8% of an eclogite mixture composed of 0.57 clinopyroxene–0.43 garnet at intermediate pressure (20 kbar range), from the 0.0125 remaining liquid fraction (thus leaving a 7.8×10^{-4} residual liquid fraction); finally followed by 50% of a gabbro mixture composed of 0.17 olivine–0.33 clinopyroxene–0.5 plagioclase at low pressure (<10 kbar range).

Figure 4 shows that such a massive fractional crystallisation scheme would change the Ge/Si, Ga/Al and Ge/Ga ratio by a far greater amount than was actually observed. The Ga and Ge crystal/melt partition coefficients used for the reconstruction in the eclogite facies (Table 2), are based on the Robert Victor mine inclusion data reported in Table 1, whereas for Si and Al they are based on the mineral compositions reported by Philpotts *et al.*²⁷ and a melt composition assumed equal to 48% for SiO₂ and 14% for Al₂O₃. Although other extraction paths on the Yb versus La/Sm diagram could have been designed (see +D-C in Fig. 4a), all seem to change the Ga/Al, Ge/Si and Ga/Ge far too much compared with the variations observed in Fig. 4b and c. Changing the Ga, Ge, Al and Si crystal/melt partition coefficients by a factor 2 to 3 times greater, or less, than previously considered, would not alter this conclusion. On this basis, O'Hara's explanation¹⁰ is very unlikely, and furthermore, O'Hara's high to low-pressure fractionation model from a single mantle source would not explain the Sr and Pb isotopic composition variation observed^{4,5}. Thus, we reject such a model as being the primary cause of the distinct Ge/Si and Ga/Al populations observed along the profile.

Mantle mixing beneath the Reykjanes Ridge

Binary mantle plume–LILE-depleted asthenosphere mixing beneath the Reykjanes Ridge, outwards from Iceland, has been suggested on the basis of La/Sm (ref. 1) and radiogenic Pb and Sr compositional variations^{4,5}. We have briefly noted that contrary to La/Sm and radiogenic Pb, Ge/Si, Ga/Al and Ge/Ga profiles do not show a clear transition zone (Fig. 2). The width of the transition zone for ⁸⁷Sr/⁸⁶Sr is intermediate between these two extremes (and depends heavily on how much weight is given to individual values). Discrepancies between such isotope and elemental ratio profiles remain puzzling. Detailed geochemical tests for binary mantle mixing, such as discussed by Schilling²⁸, Sun *et al.*⁵ and Langmuir *et al.*²⁹, have been further attempted using Ge/Si, Ga/Al and Ga/Ge, but found to be inconclusive.

Possible causes for such discrepancies are: (1) large analytical uncertainties, compared to that of the existing range of variations, produce too much scatter (such as, Ge/Si and Ga/Al, ⁸⁷Sr/⁸⁶Sr); (2) some elemental or isotope ratios, affected more than others by processes occurring after mixing (such as partial melting and fractionational crystallisation, or seawater interaction), alter in irregular and unpredictable ways the original trends produced by mantle mixing; (3) smaller scale heterogeneities may exist within these two major mantle sources (Iceland mantle plume and LILE-depleted asthenosphere), thus adding additional complexities and scatter to the simple binary mixing trends predicted; (4) insufficient and non-uniform data base makes the correlation statistically unsound (some isotope or elemental ratios are available for one sample but not for another, and non-uniform sampling density along the profile); finally, (5) it should also be emphasised that the different curvatures of hyperbolic curves for binary mixing (see ref. 29) may give the illusion of a wider, or narrower, transition zone, especially if additional noise is also introduced by some of the factors discussed above.

Conclusions

The following points should be stressed: first, the Ge/Si and Ga/Al data presented here emphasise the distinct chemical nature of the mantle present beneath Iceland compared to that of the so called LILE-depleted asthenosphere present beneath the Reykjanes Ridge south of 61°N. Melting at higher pressure beneath Iceland compared to the ridge is also suggested on the basis of SiO₂ content.

Second, the analytical uncertainties on the Ge/Si and Ga/Al ratios are too large relative to the range of variation observed along the profile, and the four elements too susceptible to small fractionation caused by partial melting and fractional crystallisation conditions, to test further and with sufficient confidence the model of binary mantle mixing previously proposed^{1,4,5}.

Finally, the exact cause of the mantle heterogeneity present beneath the Iceland–Reykjanes Ridge segment of the Mid-Atlantic Ridge remains one of the important unsolved problems in solid-Earth geochemistry. The geochemical differences between these two mantle sources of basalts cannot simply be related to volatility as first pointed out by Hutchinson³⁰. Both refractory elements (such as La, Ba, Sr (refs. 1, 31)) are enriched to similar extents as more volatile elements such as K (ref. 1), Rb, Cs (ref. 31), or highly volatile elements, such as Pb (ref. 5), F (ref. 32), Se (ref. 33) and apparently Cl and Br (ref. 34). On the other hand, Ge, which has a similar volatility to Ga (ref. 35) is depleted in the Iceland mantle relative the LILE-depleted asthenosphere, whereas Ga is enriched. No simple correlation between degree of enrichments (or depletion) and siderophile tendencies among elements such as Ga, Ge, Au, Pt or Pd could be found in a parallel study. Mantle fractionation processes involving crystals and melts, or metamorphic reactions involving release and interstitial transport of fluids, are more likely to be the cause of such heterogeneities²³.

We thank C. K. Unni and D. G. Johnson for technical assistance, F. DiMeglio and his staff of the Rhode Island Nuclear Science Center for neutron irradiations and facilities, and M. Zajac and P. Aldrich for assistance. This research was supported partly by UNESCO (fellowship to R.M.A. to attend the University of Rhode Island), the University Federal of Bahia, Brazil, and the NSF.

Received 6 June; accepted 15 September 1978.

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Planktonic foraminiferal biostratigraphy in the Equatorial Pacific

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Planktonic foraminiferal datums for the Pleistocene of the Equatorial Pacific have frequently been shifted vertically by dissolution and bioturbation. Evolutionary initial appearances and final extinctions are altered to first and last lithostratigraphic occurrences, respectively. Shallow cores with high sedimentation rates are least affected by these processes and provide the most reliable chronostratigraphic resolution. Ten datum levels are proposed for the Quaternary and five for the latest Pliocene, all of which have been dated by palaeomagnetic stratigraphy.

DATUM LEVELS based on abrupt evolutionary initial appearances or final extinctions of planktonic foraminifera have been proved useful for dating and in the correlation of deep-sea sediments¹⁻³. Although late Tertiary Pacific sediments often show intense action by dissolution, the relative succession of datum levels does not seem to have been adversely affected³. There is, however, evidence that the chronostratigraphic position of many levels relative to the palaeomagnetic record of the same cores also may have been shifted vertically in response to bioturbation^{4,5}. These two influences operate to modify primary biologically determined patterns into taphonomically determined ones: evolutionary 'initial appearances' and 'final extinctions' are modified into lithostratigraphic 'first occurrences' and 'last occurrences', respectively. Examination of widely separated sequences which, in theory, should be isochronous can occasionally be shown to be time-transgressive^{6,7}; in reality the problem may be one of local differential preservation and sedimentation.

Equatorial Pacific sediments

Planktonic foraminiferal biostratigraphy for the Late Tertiary of the Equatorial Pacific has been based on datum levels established in the Eastern Equatorial Pacific³, an area noted for its prominent bioturbation and carbonate dissolution⁸. Close scrutiny of the figures of Hays *et al.*³ showing planktonic foraminiferal occurrences often fails to show the same lithostratigraphic positions relative to the palaeomagnetic records from core to core, even within the same area. Despite this problem, however, it was possible to create a durable composite biostratigraphy.

Climatic studies conducted by the CLIMAP Project⁹ have located four important Equatorial Pacific cores: V19-96, V28-238 and V28-239 from the Ontong Java Plateau¹⁰⁻¹², and V28-179 from the eastern Equatorial Pacific^{13,14}. Here we present their biostratigraphic, palaeomagnetic, dissolution and

calcium carbonate records (Table 1; Fig. 1), and the results can be compared with those of Hays *et al.*³.

The climatic significance of the calcium carbonate records of V28-238 and V28-239 has been shown to be inconclusive¹⁰⁻¹², whereas V19-96 and V28-179 clearly show the classic pattern of alternating low and high (60-80%) carbonate percentages, interpreted as indicating glacial productivity and interglacial dissolution, respectively^{3,8}. In the lower part of V28-179, however, the average calcium carbonate content is 30-40%, suggesting warmer conditions than occurred during the Pleistocene. Oxygen isotope study of this core^{14,15} reveals a trend towards increased Quaternary cooling beginning just below the Mammoth event (1,680 cm). These two lines of data are taken to depict the onset of Late Tertiary glacial events in the Northern Hemisphere.

Biostratigraphic refinements

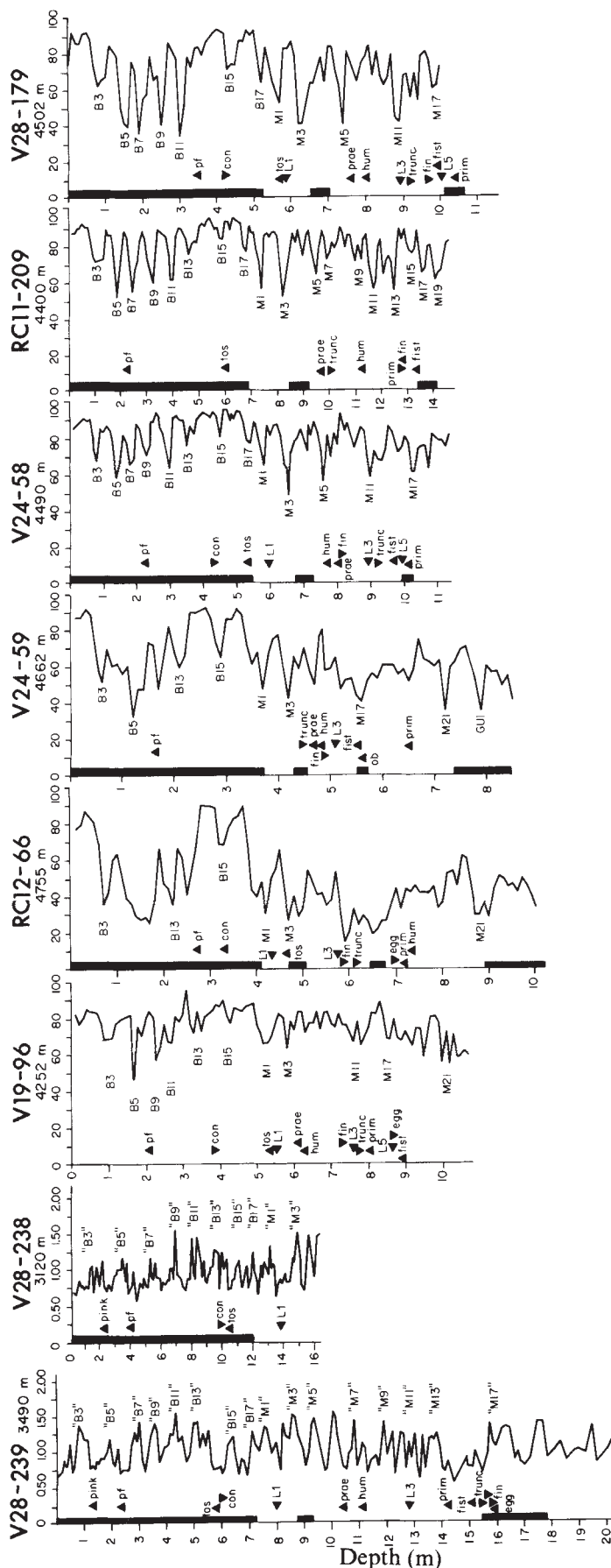
The coiling directions of *Pulleniatina* have been examined in the four new cores. The patterns which have been found are in accord with those presented for other cores of the Indo-Pacific regions¹⁶. Left-coiling peaks 1, 3, and 5-6 are well-defined but peak L2a often appears only in higher sedimentation rate cores. There seems to be little correlation of the coiling directions of *Pulleniatina* with climatic events indicated by oxygen isotopes or calcium carbonate (Figs 1, 2). The only general correlation which can be made is that the coiling directions are dominantly dextral in long normally magnetised intervals such as the Brunhes and Gauss and dominantly sinistral in long, reversely magnetised intervals such as the Matuyama and the Upper Gilbert (see Fig. 2 of ref. 16). The explanation for this observation is not clear.

Major portions of the Pleistocene to Recent interval of the Equatorial Pacific can be characterised by their planktonic foraminiferal content. Planktonic foraminiferal stratigraphic occurrences have been plotted against the coiling direction changes of *Pulleniatina*¹⁶ and against a time scale derived from the palaeomagnetic and the carbonate records of V28-239 and RC11-209 (Fig. 2). Several new biostratigraphic events have been recognised, but often cannot be recognised in some of the deeper more poorly preserved cores. These events, however, do contribute to a more exact palaeontological subdivision of a core when they can be found. Closely spaced sampling does not reveal radical changes in the fauna amenable to a formal zonation although five subdivisions (not intended as zones) permit a rough age determination and may be described briefly as follows (in increasing geological age from the present).

(1) Recent sediments contain species recovered in plankton tows but rarely observed much below the tops of the cores, sometimes including forms such as *Globigerinella adamsi* (Banner and Blow), *Hastigerinopsis digitiformans* Saito and

Table 1 Locations and water depths of cores

Core	V19-96	V24-58	V24-59	V28-179	V28-238	V28-239	RC11-209	RC12-66
Lat	00°52'S	02°16'N	02°34'N	04°37'N	01°01'N	03°15'N	03°39'N	02°37'N
Long	172°00'E	141°40'W	145°32'W	139°36'W	160°29'E	158°11'E	140°04'W	148°13'W
Depth (m)	4,252	4,490	4,662	4,502	3,120	3,490	4,400	4,755



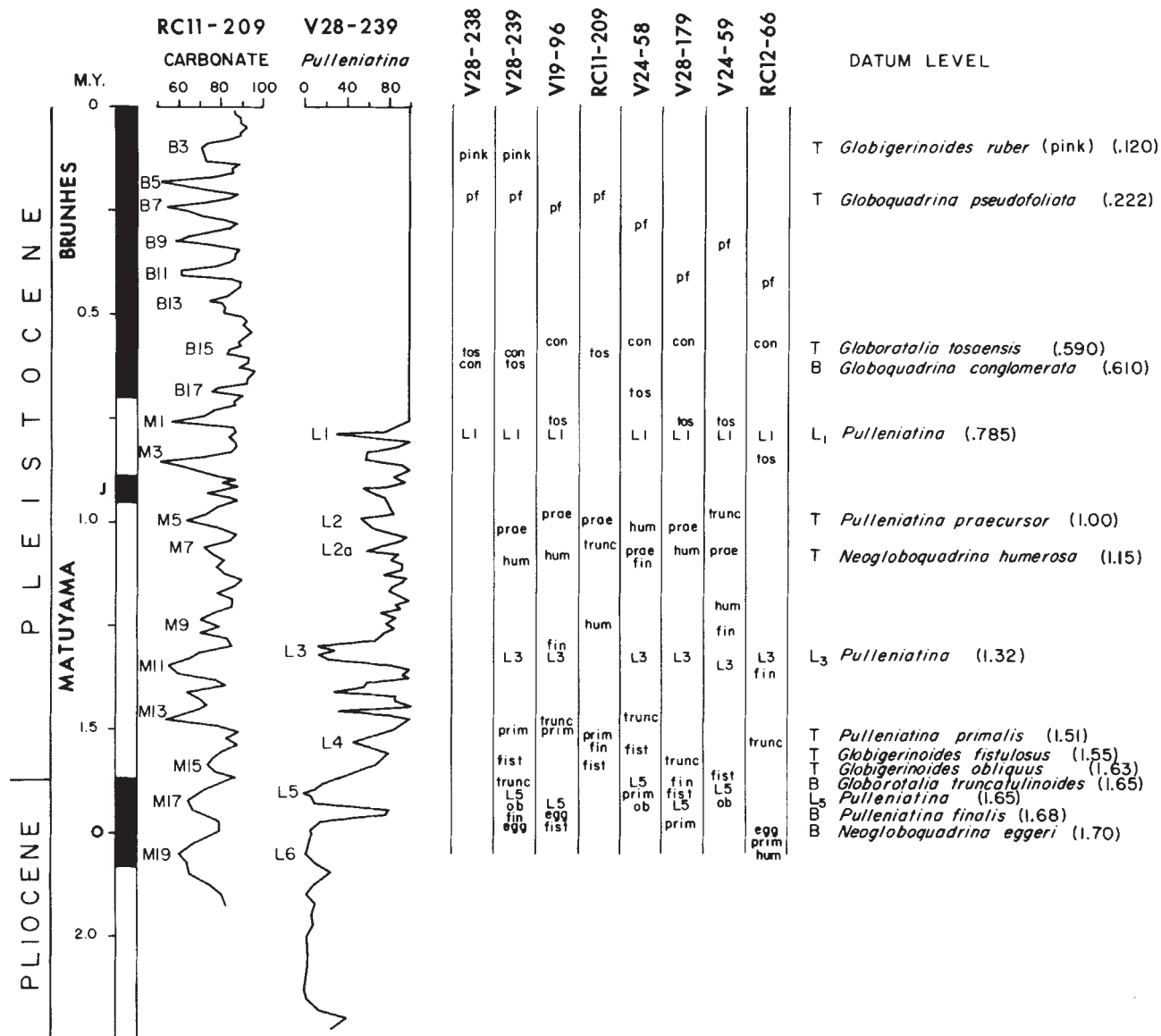
than the oxygen isotopic record for the same cores¹⁰⁻¹³. It has been suggested that all these variations probably resulted from glacially intensified circulation and nutrient upwelling at the Equator which would cause increased productivity²⁵. The net effect of dissolution with increased water depth is the differential destruction of certain species of planktonic foraminifera and the relative concentration of others²⁶⁻²⁸, leading ultimately to the elimination of all tests at the calcium carbonate compensation depth²⁹. Obviously, datum levels based on dissolution-susceptible species are not as likely to be located in cores which have undergone severe dissolution as those datums based on more resistant taxa. Further, since dissolution operates by reducing the absolute abundances of a given species from its concentration in the living assemblage relative to that of the sediment assemblage³⁰, we can expect the numerically rare individuals present at the times of an evolutionary appearance or extinction to be destroyed except where the sediments have been very well preserved^{4,5}. Chronostratigraphically determined 'initial evolutionary appearances' and 'final extinctions' become taphonomically determined higher 'first stratigraphic occurrences' and lower 'last occurrences', respectively. Graphically, whereas the lithostratigraphic position of a hypothetical abun-

dance curve (Fig. 3a) is not shifted vertically by dissolution, it typically shows (1), a marked reduction in absolute abundances and (2), the lithostratigraphic duration of the curve is shortened (Fig. 3b).

Vertical mixing

Sediment mixing in deep-sea cores is considered to be caused by the burrowing and foraging activities of benthic organisms^{4,5,6}. In cores, this effect often appears as contrasting blotches of sediment having a tubular form in space. Material from previously deposited sediments is excavated by the activity, and younger material later may fill the space when it is abandoned^{34,35}. In cores where this effect is prominent, the stratigraphy can be expected to be greatly disturbed and the infilled burrows should be avoided in sampling. In cores of uniform sediment composition, burrowing is, of course, impossible to detect visually, and the sediments may range from undisturbed to completely homogenised. The extent to which vertical mixing disturbs discreet layers has been the subject of studies using instantaneous geological events as tracers including microtektites and volcanic ashes^{4,5,36}. Faunal abundances near extinction levels may be related to ecology or dissolution and thus neither

Fig. 2 Planktonic foraminiferal occurrences and coiling direction changes of *Pulleniatina* compared with palaeomagnetic time scale, carbonate curve of RC11-209 and *Pulleniatina* coiling curve of V28-239. Cores ordered by increasing water depth from left to right. Abbreviations as in Fig. 1.



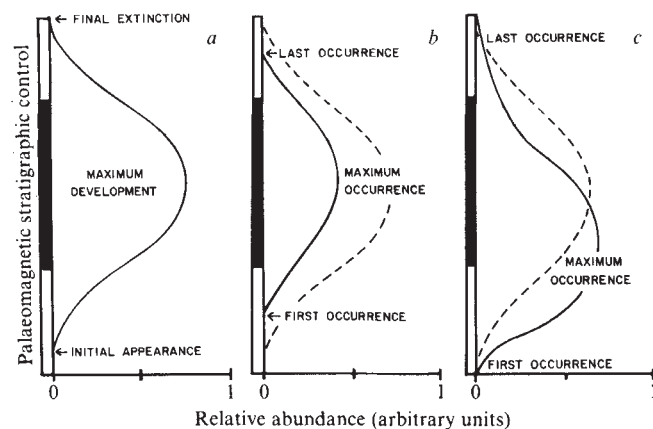


Fig. 3 Effects of dissolution and bioturbation. *a*, Hypothetical abundance curve relative to palaeomagnetic reversal sequence. *b*, Reduction in relative abundance and shortening of total range by dissolution. *c*, Extension of lithostratigraphic range and shift in position of abundance maximum by bioturbation.

confirm nor deny these models. However, we should expect that 'final extinctions' can be shifted by bioturbation to stratigraphically higher (later) 'last occurrences' (Fig. 3c). By analogy, downward mixing is also capable of moving evolutionary 'initial appearances' to stratigraphically lower (earlier) 'first occurrences'. Tektite evidence³⁶ has suggested that, because a longer 'tailing off' occurs above the peak abundance level than below it, it is easier for sediments to be mixed upwards than downwards. Thus, we may conclude that fossil appearances are apt to be more reliable chronostratigraphic indicators than are extinctions.

Discussion

The lithostratigraphic position of a sedimentary particle is determined by the original rate of deposition, the degree of preservation and the extent of redeposition; its chronostratigraphic position is in relation to a datable event such as a palaeomagnetic reversal.

The deposition rate of an organism such as a planktonic foraminifera depends on its production rate³⁰, which varies seasonally, annually, and probably also through long-term climatic fluctuations. Present-day estimates of pelagic productivity may or may not be applicable to fossil sediments. The degree of preservation of calcareous microfossils has been clearly linked with water depth for core tops. That this preservation varies through time is evidenced by calcium carbonate fluctuations in the deep cores and fragmentation in shallower cores. Redeposition through bioturbation can be observed as colour contrasts in some cores and by tektite or ash dispersions in other cores, but cannot be observed in all cores. In the absence of such a tracer, there seems to be little hope of estimating the possible displacement of the fossils. Although the palaeomagnetic time framework is generally considered to provide highly accurate and unbiased reference points, the magnetic intensity change at reversals is often gradual suggesting that some fine magnetic particles may have been either mixed³ or that the magnetic field did not reverse instantaneously⁴¹.

In the present attempt to refine Pacific datum levels based on planktonic foraminifera, calcium carbonate minima have been used as additional reference points to assess the behaviour of faunal events under the influence of sedimentological processes. The carbonate curves (plotted from sedimentation rates calculated from the palaeomagnetic stratigraphy for each core) have a distinct pattern that permits the identification of correlative peaks and troughs. Since glacial stages (isotopically defined) are characterised by relatively slight dissolution (carbonate maxima), it is suggested that calcareous microfossil datums occurring during these intervals would be lithostratigraphically closer to their true chronostratigraphic level than if

they had occurred during an interglacial (carbonate minimum) stage. Further, datum levels based on dissolution-resistant taxa may be expected to be more reliable than those based on fragile forms.

Great displacements of planktonic foraminiferal datums noted in some of the cores examined indicates intense dissolution throughout both glacial and interglacial stages for deep sites. Bioturbation, although clearly visible in photographs of the cores having contrasting sediment colours, has not been able to cancel the dissolution effect on the datum levels. It is possible to observe, however, that burrowing appears to be more intense (burrows per unit surface area over the core surface) in dark-coloured units (clay-rich interglacial stages). If this is the general case, it is possible that the benthic population can subsist with less foraging effort during glacial stages when organic productivity and deposition of carbonate was greater.

There may be a connection between all the sedimentological phenomena, the large number of extinctions of warm water taxa since the Olduvai, and the increasing intensities of Pleistocene climatic fluctuations. There is evidence that conditions dominated by burrowing (lower 'first occurrences' relative to increasing water depth) gradually gave way to dissolution dominated (higher 'first occurrences') between the Gauss and the Brunhes. The increasing tendency for dissolution to alternate with relatively better preservation implies periodic circulation intensifications in accord with the models of Equatorial Pacific carbonate sedimentation^{3,8}. If interglacial conditions are the norm in the Quaternary Pacific, then dissolution must also be the norm, and it is the glacial (productivity) increases in carbonate deposition that are atypical. The resolution of the biostratigraphy clearly has been affected by these sedimentary processes, and should not be interpreted out of the context of the lithostratigraphy of the sediments. In conclusion, since the available evidence indicates that the age modifying effects of dissolution in the Quaternary sediments of the Pacific are much more intense than those of redeposition processes, the planktonic foraminiferal biostratigraphies and the derived chronostratigraphy have the greatest accuracy in cores from hydrographically shallow depths where dissolution is minimal and deposition rates have been high enough to limit drastic bioturbation. The lithostratigraphic position of calcareous datums provides important clues to the dissolution history of the Quaternary which must be understood before either age or climatic interpretations can be made.

We thank L. H. Burckle and W. F. Ruddiman for reviewing the manuscript, T. Saito for permitting us to re-examine his samples, and D. B. Ericson and G. Wollin for providing unpublished data on V19-96. This research was supported by the NSF Office of Climate Dynamics and IDOE grants GX28671 and OCE75-19627, and by NSF grant OCE76-18049 and Naval Research grant N00014-75-C-0210 to the Lamont-Doherty Geological Observatory Core Laboratory of Columbia University.

Received 2 August; accepted 24 August 1978.

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Identification of a single promoter in *E. coli* for *rplJ*, *rplL* and *rpoBC*

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*A set of restriction fragments cloned into phage λ vectors has allowed us to locate a site necessary for full *rpoBC* expression. We find that these genes for the two large subunits of RNA polymerase and the genes, *rplL* and *rplJ*, for two ribosomal proteins, form a single operon.*

THE RNA polymerase of *Escherichia coli* K12 is an oligomeric enzyme of at least four subunits, α , β , β' and σ (ref. 1). The genes for β and β' , termed *rpoB* and *rpoC* map together on the bacterial chromosome at position 88.5 min (ref. 2). They are transcribed together in the order B $\rightarrow$ C (refs 3-5). Upstream from *rpoBC* lies a cluster of genes concerned with protein synthesis (Fig. 1a). They include a ribosomal RNA gene (*rriB*), and genes for several tRNAs, EF-Tu (*tufB*), and the ribosomal proteins L11 (*rplK*), L1 (*rplA*), L10 (*rplJ*) and L7/L12 (*rplL*)⁶⁻⁸. The evidence^{9,10} suggests that these genes are read in the same direction as *rpoBC*, thus raising the possibility that they share a common promoter. The evidence presented here shows that the genes *rpoBC* are indeed co-transcribed with the two ribosomal protein genes immediately upstream: *rplL* and *rplJ*. Thus we have demonstrated a mechanism relating β and β' synthesis to ribosome synthesis.

Sequential cloning of the *rpo-tufB* region

The approach we have adopted to locate the *rpoBC* promoter is to determine how much of the adjacent DNA must be jointly excised with *rpoBC* in order to create a DNA molecule capable of independent *rpoBC* expression. We have cloned different-sized restriction fragments of the *rpo-tufB* region into λ vectors¹¹ and have studied expression of the recombinant phages in UV-irradiated bacteria.

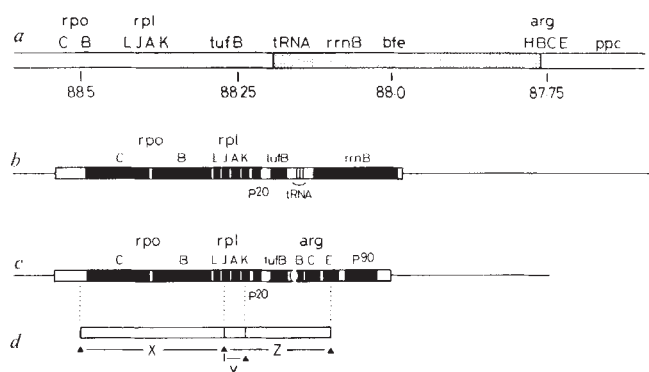
The bacterial DNA carried by λ *drif*^d18 has been intensively analysed (Fig. 1b)^{6,12,13}. These studies locate the restriction sites which we have used to excise separate fragments containing the *rpo-tufB* region. In our own studies we have not used λ *drif*^d18 as a source of these fragments. Instead we use λ *darg* Δ M9 (Fig. 1c) which derives from λ *drif*^d18 but contains a deletion, Δ M9, which arose on an F' factor KLF10. The deletion removes the DNA from *argB* through *rriB* past the promoter for *tufB* (Fig. 1a) thus placing *tufB* expression under arginine regulation¹⁴. To transfer the deletion to a transducing phage we first inserted λ *drif*^d18 into the *rpoBC* genes of KLF10 Δ M9 by a reciprocal crossover in a *rec*⁺ bacterium. We then transferred the KLF10 Δ M9 λ *drif*^d18 obtained to a *recA* bacterium in which the new phage arose by an aberrant excision¹⁴. It was selected as a phage able to transduce to *argE*⁺ and carries both *argEC* and the *tufB-rpoBC* segment. The restriction fragments obtained

from λ *drif*^d18 and λ *darg* Δ M9 (Fig. 2) are drawn in Fig. 3. Note that both phages generate the major *Hind*III fragments (11.2 and 21.0%) which contain the *rpoBC* extremity of the bacterial DNA but that the large 27.9% fragment of λ *drif*^d18 is replaced in λ *darg* Δ M9 by two shorter segments (12.6% and 14.8%) due to the deletion Δ M9.

The fragments which we are particularly concerned to clone are shown in Fig. 1d. The large *Hind*III fragment, X (21.0%; Fig. 3, λ *darg* Δ M9), contains *rpoBC* and *rplL*. Fragment Y lies immediately upstream from *rplL*. It shares a *Hind*III site with fragment X to the left and has an *Eco*RI site to the right. Finally fragment Z (12.6%; Fig. 3, λ *darg* Δ M9), obtained from *Hind*III digests, contains fragment Y extending further upstream to include *tufB* and *argC*.

Our fragments were cloned into phage λ vectors designed by Murray *et al.*¹¹. One of these, λ 761, is a replacement vector for *Hind*III fragments. It carries the amber suppressor *supF* (heavy

Fig. 1 The *rpo-rpl* genes investigated and their neighbours. a, The chromosomal segment. Numbers below the line are map coordinates in minutes⁷. The DNA deleted by Δ M9 is shown stippled. b, λ *drif*^d18. Phage DNA is shown as a thin line; the entire right arm from *att* is present in this phage (see Fig. 3). The bacterial DNA contains a mutation, *rif*^r, (not shown) in *rpoB*, determining dominant rifampicin resistance. c, λ *darg* Δ M9. Less phage DNA (thin line) survives in this phage; the genes from *att* through N (see Fig. 3) have been deleted. The bacterial insertion includes genes from both sides of Δ M9 (shown as parentheses). d, Restriction fragments involved in this study. They are more fully documented in Fig. 3. X is the 21.0% *Hind*III fragment of λ *drif*^d18 or λ *darg* Δ M9; Y is a 3.8% fragment resulting from endo R.*Hind*III restriction of the 4.4% *Eco*RI fragment of λ *darg* Δ M9; Z is the 12.6% *Hind*III fragment of λ *darg* Δ M9.



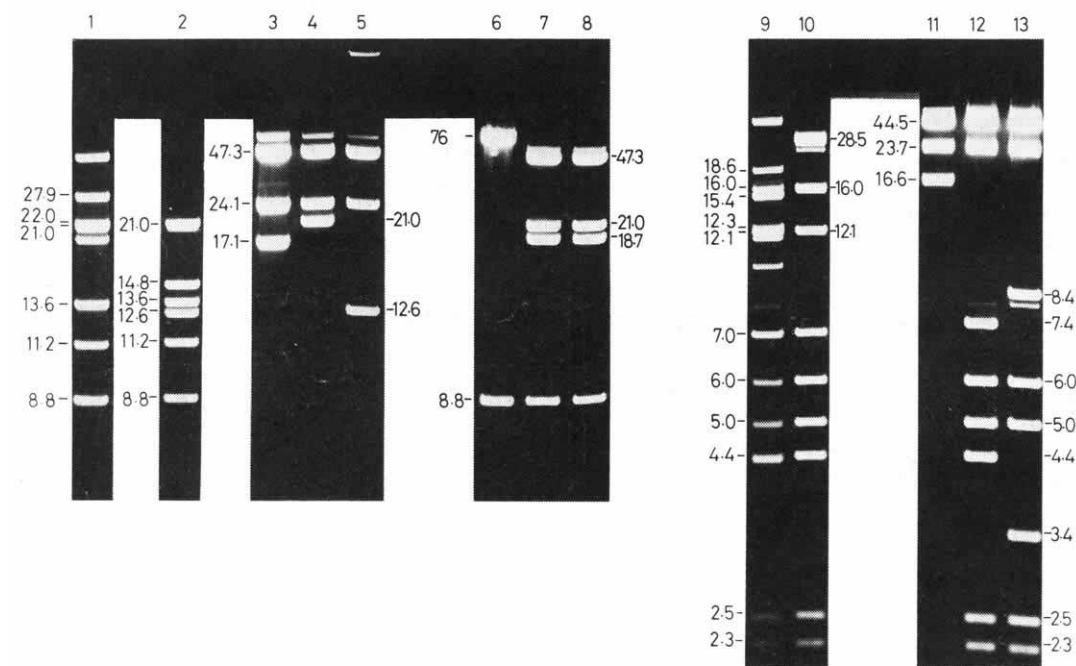


Fig. 2 The restriction enzyme analyses of transducing phage DNA. Tracks 1-8 contain phage DNAs digested with endo-*R.Hind*III and analysed by electrophoresis on a horizontal 0.7% agarose gel.^{11,17} 1, λ drif^d18; 2, λ darg Δ M9; 3, NM λ 761; 4, λ p(*rpl-rpo*)1; 5, λ p(*arg-rpl* Δ M9); 6, NM λ 791; 7, λ p(*rpl-rpo*)76; 8, λ p(*rpl-rpo*)24. Tracks 9-13 contain phage DNAs digested with endo *R.Eco*RI and electrophoresed on a 1.0% agarose gel.¹¹ 9, λ drif^d18; 10, λ darg Δ M9; 11, NM λ 791; 12, λ p(*rpl-rpo*)76; 13, λ p(*rpl-rpo*)24. The gels were stained with ethidium bromide and photographed under UV light.

The sizes of the resultant fragments are given as % of wild-type λ DNA and are based on the data of refs 6, 13, 27, unpublished data of P. Philippsen and R. W. Davis, and our own work. The sizes of some of the fragments from λ drif^d18 are slightly different from those originally given by Lindahl *et al.*⁶; however, the sizes presented here are consistent with the size of wild-type λ DNA fragments given by others (ref. 28, Philippsen and Davis). The minor unlabelled bands in the λ drif^d18 samples are from the contaminating wild-type λ helper DNA. The 1.2% and 0.2% *Hind*III fragments of λ drif^d18 and λ darg Δ M9 are not shown on this gel. NM 761 and NM 791 are replacement vectors for *Hind*III and *Eco*RI restriction fragments respectively¹¹.

line) inserted between two *Hind*III targets (Fig. 3). As all other *Hind*III targets have been removed from λ 761, a digest of this phage releases only three fragments—the left and right arms of λ , and *supF*. Because DNA has been extensively deleted from the two arms of λ 761 a viable phage can only reform by incorporating either the *supF* fragment or another suitable *Hind*III fragment added to the digest¹¹. After reannealing and ligation the *supF* fragment can be distinguished by plating transfected *lacZ* amber bacteria on lactose MacConkey indicator medium¹¹. Phages reincorporating *supF* give red plaques with Lac⁺ cells at the centre. New phages with *supF* replaced by different DNA give white Lac⁻ plaques. Mixed *Hind*III digests of λ darg Δ M9 and λ 761 yielded about 54% Lac⁻ plaques. The fragments cloned into these phages include X, Z and the rightward extremity of the bacterial DNA carrying the *ppc* gene (not relevant to the present study). Phages carrying X (20% of all recombinants), such as λ p(*rpl-rpo*)1, are able to transduce a *rec*⁺ bacterium to rifampicin resistance showing that they inherit the resistance mutation in *rpoB*, carried by λ darg Δ M9 and originating in λ drif^d18 (ref. 15). Phages carrying Z (19% of all recombinants) can transduce to *argC*⁺ (λ p(*arg-rpl* Δ M9)). Their structure (Fig. 5a, b) is confirmed by restriction analysis showing that they generated X and Z respectively (Fig. 2).

From recent evidence¹⁶ we were aware that fragment X would carry *rpoBC* intact and *rplL*, and that it probably lacks the *rpoBC* promoter. We therefore undertook to construct a phage incorporating both X and Y.

The composite phage was made by mixing a *Hind*III digest of λ p(*rpl-rpo*)1 (to donate X and the left arm of λ), purified fragment Y, and an *Eco*RI digest of the *Eco*RI replacement vector, λ 791, which when intact gives blue Lac⁺ plaques on indicator medium containing 5-bromo-4-chloro-3-indolyl- β -D-galactoside (Fig. 3). This provided the right arm of the phage. Fragment Y (3.8%) was purified on a 1.0% agarose gel from a double *Hind*III/*Eco*RI digest of λ darg Δ M9 DNA. The desired region was excised from the gel, the DNA electroeluted¹⁷ and concentrated by ethanol precipitation. The appropriate bacteria were transfected by the ligated mixture and plated for plaques on indicator plates. Recombinant phages make white Lac⁻ plaques. Amongst these we sought phage carrying *rif*^r (on

fragment X) and the phage marker *imm* λ characteristic of the right arm of λ 791 (Figs 3 and 5). Phages with both these characters should automatically carry fragment Y, joining the *Hind*III site of fragment X to the *Eco*RI site of the right arm.

Phages of this type (representing 65% of the *imm* λ *lac*⁻ plaque formers) do carry Y as expected. We show *Hind*III and *Eco*RI digests of two such phages (λ p(*rpl-rpo*)76 and λ p(*rpl-rpo*)24). Both contain fragment X (Fig. 2, tracks 7 and 12; 8 and 13). One of them, λ p(*rpl-rpo*)76, has X in the correct orientation since *Eco*RI digests regenerate the parental 4.4% fragment (compare tracks 12 and 10, Fig. 2). The other λ p(*rpl-rpo*)24 does not, implying that its X fragment is in the reverse orientation (compare tracks 13 and 10, Fig. 2). The restriction analysis of these phages is summarised in Fig. 3.

Gene expression in *rpo-tufB* fragments

A characteristic set of ³⁵S-labelled proteins is made in UV-irradiated *lysogenic* bacteria¹⁸ following infection by our phages (Fig. 4). They can be easily identified on SDS gels by reference to the proteins of the parent phages, λ (track 1), λ drif^d18 (track 2) and λ darg Δ M9 (track 3).

In the λ -immune lysogenic host only two λ proteins are synthesised: the repressor and *rex* proteins (track 1). By contrast, the bacterial genes on λ drif^d18 and λ darg Δ M9 (tracks 2 and 3) are clearly expressed and their products appear on the gels in the following size order: β' and β subunits of RNA polymerase, EF-Tu, ribosomal proteins L1, P20 (an unidentified protein), L10, L11 and L7/L12. Note that λ darg Δ M9 makes EF-Tu at a much lower rate than λ drif^d18. This is because *tufB* is under *arg* repression in λ darg Δ M9¹⁴. It also makes the *argE* and *C* products and a 90,000 MW protein, possibly the *ppc* gene product (track 3).

The phage λ p(*rpl-rpo*)1 contains fragment X alone. It reproducibly makes a small amount of β and β' in lysogenic cells representing at most 1–2% of that made by λ drif^d18 and λ darg Δ M9 (Fig. 4, tracks 4, 2 and 3). At the same time we can be sure that this phage does carry intact *rpoB* and *rpoC* as on infecting non-lysogenic cells it makes large amounts of β and β' due to transcription from the λ promoter P_L (track 8)^{11,29,30}. Indeed in non-lysogenic cells it also makes the protein L7/L12,

confirming that fragment X carries an intact copy of *rplL*¹⁶. We conclude that fragment X lacks a sequence, presumably including the promoter, which is present in λ drif^d18 and λ darg Δ M9 and is necessary for normal *rpoBC* and *rplL* expression.

The phage λ p(*rpl-rpo*)76 containing X and Y provides positive confirmation of this conclusion. It directs synthesis of β , β' and L7/L12 in equivalent amounts to the parent phages in lysogenic cells (track 6). We infer that fragment Y contains the necessary sequence. Furthermore λ p(*rpl-rpo*)76 must also contain and express *rplJ*, as it can be seen to direct synthesis of

Fig. 3 Restriction site map of transducing phages. The targets for endo R.*Eco*RI are shown by arrowheads above the maps and those for endo R.*Hind*III below. The size of the fragments are given as the % of wild-type λ DNA. The bacterial DNA inserted into each phage appears as a thick line. The maps for λ (refs 27, 28) λ drif^d18 (refs 6, 13) NMA 761 and NMA 791 (ref. 11) λ darg Δ M9 (ref. 14) and a phage similar to λ p(*rpl-rpo*)1 (ref. 16) have been previously determined. Small size adjustments for some fragments are explained in Fig. 2. The *Hind*III replacement vector NMA 761 and its derivatives λ p(*rpl-rpo*)1 and λ p(*arg-rpl* Δ M9) carry the immunity region of the lambdoid phage 21 (*imm*21). The other phages have λ immunity with a *ts* mutation (cl857) in the repressor gene (λ drif^d18; λ darg Δ M9) or a deletion (λ clKH54; ref. 11). The extra 0.2% endo R.*Hind*III fragment present in λ drif^d18 and λ darg Δ M9 is generated by the cl857^{ts} mutation in these phages. The 21.0% *Hind*III fragment of λ p(*rpl-rpo*)1, λ p(*rpl-rpo*)76, and λ p(*rpl-rpo*)24 corresponds to X in Fig. 1. The unshaded segment of λ p(*rpl-rpo*)76 and 24 is fragment Y (Fig. 1), a 3.8% fragment produced from λ darg Δ M9 by double digestion with endo R.*Eco*RI and endo R.*Hind*III. Although the endo R.*Hind*III maps of λ p(*rpl-rpo*)76 and λ p(*rpl-rpo*)24 are identical the fragments produced by endo R.*Eco*RI digestion allow us to deduce that they carry the 21.0% *Hind*III fragment in opposite orientations. Endo R.*Eco*RI digestion of λ p(*rpl-rpo*)76 generates the 4.4% fragment originally found on λ darg Δ M9 or λ drif^d18, while digestion of λ p(*rpl-rpo*)24 does not and thus contains the 21.0% fragment in the inverse orientation to that found on the chromosome.

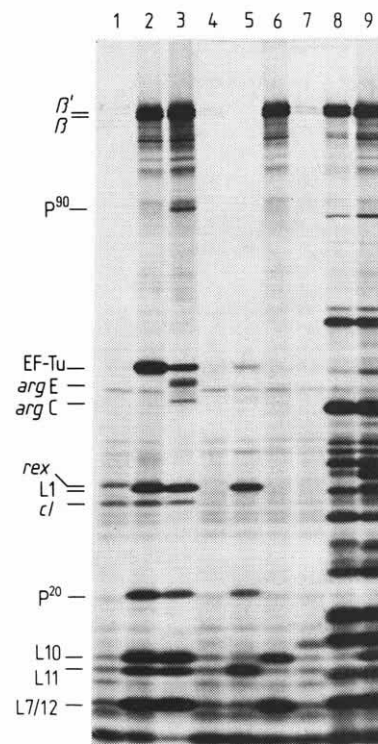
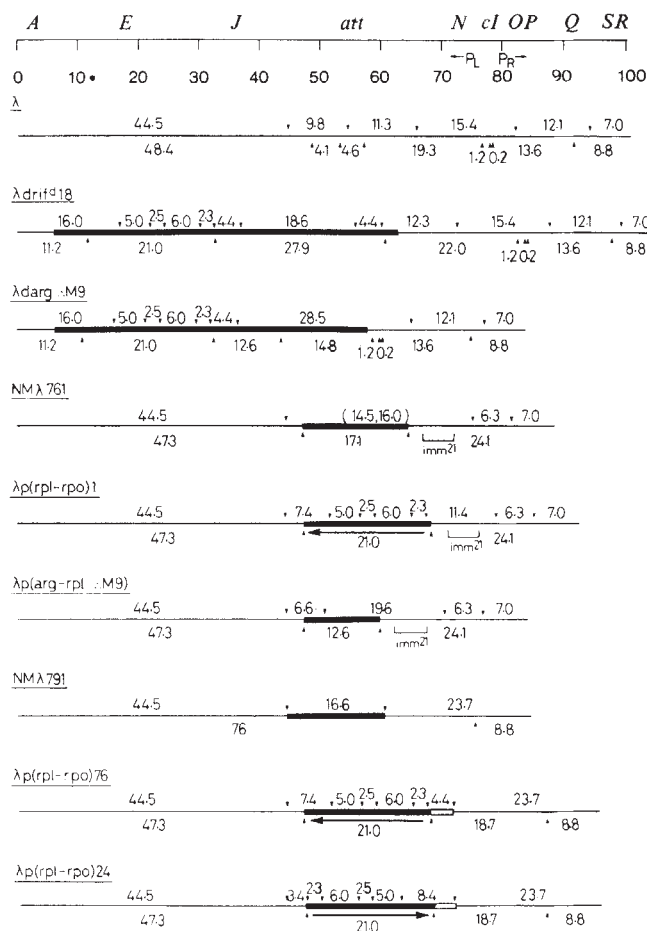


Fig. 4 Phage-directed protein synthesis in UV-irradiated bacteria. We compared protein synthesis in lysogenic (λ^{imm+} *ind*⁻ or λ^{imm21}) and non-lysogenic *E. coli* 159 (*uvr gal str-r*). Bacteria (10^9 per ml) were heavily UV-irradiated ($4,500 \text{ erg mm}^{-2}$) and infected with purified transducing phage ($\text{MOI} = 10$)¹⁸. After 20 min the infected cells were labelled for 3 min with ³⁵S-methionine (290 Ci mmol^{-1}), chased with cold methionine (4 min) and lysed for analysis by SDS-acrylamide gel electrophoresis and autoradiography^{14,16}. Lysogenic bacteria repress synthesis of all proteins except the λ cI (repressor) and *rex* products and the products of bacterial genes whose promoters are retained on the infecting transducing phage. Non-lysogenic bacteria give the above proteins plus the remaining phage gene products and the products of bacterial genes able to be transcribed by read-through from phage promoters^{29,30}. Background protein synthesis by the UV-irradiated bacteria alone can best be seen in track 1, which otherwise only contains the *rex* and *cI* gene products. Lysogenic hosts: 1, 159 (λ^{imm+}) plus λ^+ gives *rex* and *cI* gene products. 2, 159 (λ^{imm+}) plus λ drif^d18 gives β' , β , EF-Tu, L1, a 20,000 MW protein (P^{20}), L10, L11 and L7/L12. 3, 159 (λ^{imm+}) plus λ darg Δ M9 gives the same proteins as λ drif^d18, a 90,000 MW protein (P^{90}) and the *argE* and *argC* gene products.¹⁴ EF-Tu is made in lesser amounts because it is under *arg* regulation¹⁴. 4, 159 (λ^{imm21}) plus λ p(*rpl-rpo*)1 gives small amounts of β' and β only. 5, 159 (λ^{imm21}) plus λ p(*arg-rpl* Δ M9) gives EF-Tu, *argC* product, L1, P^{20} and L11. 6, 159 (λ^{imm+}) plus λ p(*rpl-rpo*)76 gives β' , β , L10 and L7/L12. 7, 159 (λ^{imm+}) plus λ p(*rpl-rpo*)24 gives only small amounts of β' and β . Non-lysogenic host (159 λ^-): 8, λ p(*rpl-rpo*)1 gives increased β and β' and L7/L12. 9, λ p(*rpl-rpo*)76 gives L1 in addition to proteins made in the non-lysogen (track 6). Note, λ p(*arg-rpl* Δ M9) does not give L10 in 159 λ^- (data not shown).

L10. Finally we can be sure that the expression we observe requires transcription from fragment Y into X because the sister phage λ p(*rpl-rpo*)24 which has X in the reverse orientation is unable to express these genes (track 7).

Is the promoter for *rpoBC* and *rplL* shared with *rplJ*? A direct answer to this question is provided by the properties of λ p(*arg-rpl* Δ M9)1 which contains fragment Z (track 5). As expected it directs synthesis of EF-Tu (under *arg* control) and the *argC* protein, L1, the protein P^{20} and L11. Most significantly it does not make L10, implying that the leftward end of fragment Z severs *rplJ*. This end of fragment Z coincides with the boundary between X and Y in λ p(*rpl-rpo*)76, showing that the promoter for *rpoBC* and *rplL* is upstream of, and therefore shared with, *rplJ*.

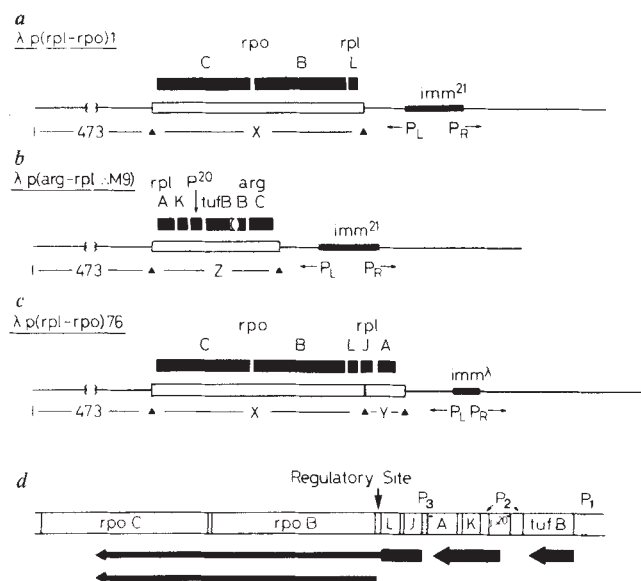


Fig. 5 Documentation of the promoters for the *rpl-rpo* genes investigated. The solid bars above *a*, *b* and *c* represent the genes whose products are made in UV-irradiated bacteria. The phage λ p(arg-*rpl*ΔM9) and λ p(*rpl-rpo*)76 both contain part of *rplJ*, severed by *Hind*III and therefore are unable to give a product in UV-irradiated bacteria. *a*, λ p(*rpl-rpo*)1, carrying fragment X cloned in NMλ761. *b*, λ p(arg-*rpl*ΔM9), carrying fragment Z cloned in NMλ761. *c*, λ p(*rpo-rpl*)76, carrying fragments X and Y cloned between the left arm of NMλ761 and the right arm of NMλ791. *d*, The pattern of transcription concluded from this study. We cannot say whether the protein P²⁰ has its own promoter or uses P2. It does not use P1 (ref. 14). The arrows show (not quantitatively) the different modes of transcription for *rpoBC* and their neighbours.

Finally our gels indicate that in fragment Y *rplA* is intact but *rplK* is not. In non-lysogenic cells λ p(*rpl-rpo*)76 does not make L11 but does make L1 (track 9). Nevertheless the promoter for *rplA* must be missing as this phage does not make L1 in lysogenic bacteria.

Thus we can now document the promoters of the *rpo-rpl* region as follows (Fig. 5d). There are two major promoters, one (P3) between *rplJ* and *rplA*, and the other (P2) upstream from *rplK*. We believe that P2 lies between *rplK* and *tufB* because evidence to be presented elsewhere¹⁴ shows that *tufB* has its own promoter (P1). Finally a limited but significant synthesis of β and β' by λ p(*rpl-rpo*)1 in lysogenic cells points to the existence of a minor promoter for separate *rpoBC* expression.

The promoter P3 is used *in vivo*

The importance of P3 for *rpoBC* expression in growing cells has been tested in the following way. A phage carrying a functional *rpoB* with the *rif^d* mutation from λ drif^d18 should make an RNA polymerase which is rifampicin-resistant¹⁵. Moreover the *rif^d* mutation is dominant, so that a lysogen carrying *rif^d* on λ and *rif^s* in the chromosomal *rpoB* gene is resistant to rifampicin. This provides a simple test for the ability of our recombinant phages to express *rpoBC* in normal growing cells, as only phages making full amounts of rifampicin-resistant enzyme should make lysogens able to grow on the drug. We compared (Table 1) two phages in this test. They have identical DNA content, but one (λ p(*rpl-rpo*)24) has *rplL rpoBC* inverted with respect to P3 whilst the other (λ p(*rpl-rpo*)76) is in the correct orientation. These phages both need a λ wild-type helper to lysogenise the host as they are integration defective and lack the repressor gene (*VcI*). As expected, in the presence of helper, λ p(*rpl-rpo*)76 gives many rifampicin-resistant colonies (Table 1). By contrast λ p(*rpl-rpo*)24 gives hardly more rifampicin-resistant colonies than the control bacteria plated alone. We conclude that in order to give expression of *rpoBC* at a level adequate to support growth these genes must be properly connected to P3.

Coordinate synthesis of RNA polymerase subunits and ribosomal proteins

Our stepwise analysis of the DNA lying upstream of *rpoBC* shows that the genes for the two large RNA polymerase subunits are jointly transcribed with the ribosomal protein genes *rplL* and *rplJ* from promoter P3 (Fig. 5d). A similar relationship between the α subunit gene (*rpoA*) and those for the ribosomal proteins S11, S4 and L17 has been previously reported¹⁹.

Clustering of the genes for ribosomal proteins and RNA polymerase subunits has been observed in widely different bacteria^{20,21}. We can now provide a biological reason for this proximity. We suggest that their co-transcription is an important general feature in the growth coordination of prokaryotes. Our results provide a solid genetic basis for the indication arising from hybridisation studies on messenger RNA from steady-state bacteria that the regulation of *rpoBC* is related to the regulation of the adjacent ribosomal genes⁴. In the light of our findings it is now important to reinvestigate this problem with separate hybridisation probes for the *rplLJ* and *rplAK* messengers.

Although transcription of *rpoBC* is initiated at P3 it is likely that the genes are partly regulated through a site between *rplL* and *rpoB* (Fig. 5d). Two important observations lead to this view. First, β and β' proteins are synthesised at a markedly lower rate than L7/L12 and L10. Second, their synthesis can be independently induced. Appropriate drug or heat inactivation of β and/or β' subunits leads to joint induction of *rpoBC*²²⁻²⁵ without *rplL*²⁶. What is the nature of the postulated regulatory site? There is a plausible model⁶ which can now be tested. It proposes that the regulatory site is an attenuator. Transcription is initiated at P3 at a high frequency to satisfy the demand for L10 and L7/L12. Many of these transcripts are terminated at the regulatory site but some (perhaps one fifth in growing cells⁴) read through to *rpoBC*. Thus *rpoBC* regulation may be realised by modulation of termination at this site.

We should also expect to find in the region of this regulatory site a sequence able to act as a low level promoter. There is no doubt that there is an independent promoter in this region, but its role in growing cells is less clear. Certainly, the evidence from lysogens suggests that this promoter alone is unable to provide polymerase subunits to support growth (Table 1; ref. 16). Finally it is worth recalling that these studies were performed on mutant DNA (*rif^d*). Since this mutation could have regulatory effects a similar study on fragment Y from a wild-type strain could be very interesting.

Since submission of this manuscript we have heard that Yamamoto and Nomura have independently performed experiments similar to ours, and have reached the same conclusion, namely that the *rpoBC* genes are co-transcribed with *rplJ* and *rplL*.

Table 1 Transduction of a *recA* strain to Rif-r by λ p(*rpl-rpo*)24 and λ p(*rpl-rpo*)76

Input phage	No. of Rif-r transductants per plate:	
	with helper λ	without helper λ
λ p(<i>rpl-rpo</i>)76	2,400	None
λ p(<i>rpl-rpo</i>)24	50	None
None	33	None

A late log broth culture of TGL10 *his trpA9761 argE171 recA56* resuspended in 0.01 M MgSO₄ was mixed with transducing phage (multiplicity of infection, MOI = 10⁻³) and, where appropriate, λ wild type (MOI = 1). The mixtures were incubated at 30°C for 30 min before plating in top agar on broth plates without rifampicin. After 6 h of incubation at 37°C to allow *rif^d* expression in the transductants the plates were overlaid with a second layer of top agar containing rifampicin (final concentration 100 μ g ml⁻¹)¹⁵. Plates were scored after 2 days. Note that the transducing phages are *VcI* and can therefore bring about significant killing on the plates in the absence of helper.

We thank Richard Hayward and Andy Newman for information supplied before publication, John Boothroyd, Michael Goman, Richard Hayward, Noreen Murray, Andy Newman and Douglas Ward for helpful discussions and for strains, and Helen Grozier for technical assistance. Lepetit Pharmaceuticals provided rifampicin. T. G. L. was supported in part by an NIH Postdoctoral Fellowship. This work was supported by an MRC grant.

Received 7 July; accepted 19 September 1978.

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Comparison of cloned rabbit and mouse β -globin genes showing strong evolutionary divergence of two homologous pairs of introns

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Cloned β -globin genes of both mouse and rabbit each contain a large and a small intervening sequence (intron) of about equal length at precisely the same positions relative to the coding sequence. The homologous introns show some sequence similarity, particularly at the junctions with the coding sequence. They most probably arose from a common ancestral sequence and diverged substantially during evolution.

ANALYSIS of several eukaryotic genes has shown that the nucleotide sequences coding for one polypeptide chain are frequently arranged in noncontiguous blocks (for a review see ref. 1). The sequences lying between these blocks, the so-called intervening sequences² or introns³, are not present in the mature mRNA; it has been shown in at least one instance that they are transcribed into a mRNA precursor and must therefore be eliminated during its processing^{4–6}.

Tilghman *et al.*⁷ have cloned a 7,000-base pair segment of mouse genomic DNA containing a β -globin gene, and have determined by R-loop hybridisation that the gene consists of at least two, perhaps three, blocks of coding sequences separated by an intron of 550 nucleotides, and possibly a second unidentified, shorter intron². The location of the large intron was determined by nucleotide sequence analysis of the junction between the coding sequence and the 5'-terminal segment of the intron². Jeffreys and Flavell⁸ have shown, by restriction site analysis of chromosomal DNA, that the rabbit β -globin gene is arranged in at least two blocks, separated by 600 base pairs. We have cloned a 5,100-base pair rabbit DNA fragment containing in its midpart a β -globin gene. To determine whether the sequences coding for the β -globin gene of the mouse and the

rabbit were interrupted at homologous positions, and to compare the nucleotide sequences of the homologous introns of the two organisms, we prepared restriction maps of the cloned mouse⁷ and rabbit chromosomal DNA and determined the sequence of several segments of interest, in particular those containing the junctions between the introns and the coding regions.

Preparation of a hybrid plasmid containing the rabbit β -globin gene and neighbouring regions

Cleavage of rabbit DNA with *KpnI* gives rise to a 5,100-base pair fragment containing in its middle portion all β -globin-specific sequences detectable by hybridisation in stringent conditions⁹. To enrich for this fragment, *KpnI*-cleaved rabbit DNA was subjected to preparative agarose gel electrophoresis, the gel was sliced and the DNA eluted from the appropriate slices¹⁰. A sample of each fraction was subjected to analytical agarose gel electrophoresis, the DNA was transferred to a Millipore membrane by the Southern technique¹¹, and the β -globin-specific DNA visualised by hybridisation to ³²P-labelled rabbit β -globin cDNA-containing hybrid plasmid P β G-1 (ref. 12) (Fig. 1). The fraction corresponding to a chain length of 4,800–5,200 base pairs showed a positive response. It contained about 1% of the original DNA; thus, the β -globin-specific DNA was enriched maximally 100-fold. The partially purified DNA was elongated with dAMP residues, hybridised to *EcoRI*-cleaved, dTMP-elongated pCRI, and transfected into *Escherichia coli* χ 1776. About 10,000 kanamycin-resistant colonies were obtained per μ g elongated rabbit DNA fragment. They were assayed by the *in situ* hybridisation procedure of Grunstein and Hogness¹³, using the ³²P-labelled 333-base pair

Table 1 Hybrid DNAs referred to in this report

Full designation	Abbreviated designation	Vector (cleavage site)	Source of cloned DNA	Insertion techniques	Ref.
Z-pCRI/Rchr β G-1	R-chrG	pCRI (<i>Eco</i> RI)	Rabbit chromosomal DNA <i>Kpn</i> I fragment	AT-tail	This paper
PMB9/Rc β G-1	P β G	pMB9 (<i>Eco</i> RI)	cDNA to rabbit globin mRNA	AT-tail	12
Z-pCRI/Mc β G-1	M-cG	pCRI (<i>Eco</i> RI)	cDNA to mouse globin mRNA (Porton Swiss mice)	AT-tail	5 and this paper
pBR322/Mchr β G-36	M-chrG	pBR322 (<i>Eco</i> RI)	λ gtWES · β G-2	Ligation	This paper*
λ gtWES · β G2		λ gtWES (<i>Eco</i> RI)	Mouse chromosomal DNA <i>Eco</i> RI fragment (plasmacytoma MOPC-49 of BALB/c mouse)	Ligation	7

Z, Zürich; R, rabbit; chr, chromosomal; m, mouse; c, cDNA; G, globin.

* λ gtWES · β G1-2 DNA was cleaved with *Eco*RI, mixed with an equimolar amount of *Eco*RI-cleaved pBR322 and incubated with DNA-ligase. Transfection was into *E. coli* χ 1776 (ref. 38). β -globin DNA-containing colonies were identified by the Grunstein-Hogness procedure¹³.

*Hae*III fragment of P β G which contains the DNA region coding for amino acids 27–138 of the rabbit β -globin polypeptide. One colony out of about 60,000 was identified as containing rabbit β -globin-specific DNA and designated as Z-pCRI/Rchr β G-1, abbreviated to R-chrG (see Table 1).

Characterisation of the hybrid plasmid R-chrG containing a segment of β -globin-specific rabbit chromosomal DNA

Unlabelled R-chrG DNA (form I) was cleaved with several restriction enzymes, and analysed by agarose gel electrophoresis (data not shown). The overall size of the plasmid, about 18,000 base pairs, was estimated by adding up the lengths of the three fragments, 8,500, 8,500 and 1,500 base pairs, obtained after digestion with *Pst*I. The largest fragment arising after cleavage of pCRI with *Hha*I had 1,400 base pairs and contains the *Eco*RI

site¹⁴; the largest fragment found after cleavage of R-chrG with *Hha*I had 6,200 base pairs. Thus, the insert consists of not less than about 4,800 base pairs (including the AT-linker segments). This value is in reasonable agreement with the length of 5,100 base pairs determined for the β -globin-specific *Kpn*I fragment excised from rabbit DNA⁹.

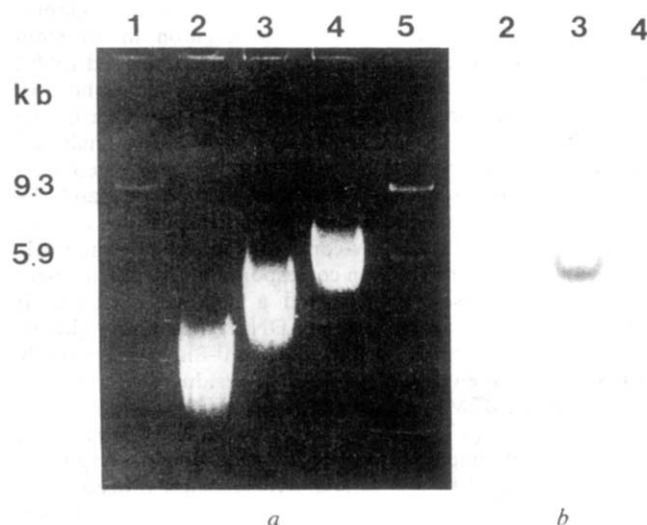
The orientation of the insert relative to pCRI was derived as follows. pCRI has one *Hind*III site and no *Bam*HI site. Double digestion of the chromosomal DNA plasmid with *Bam*HI and *Hind*III yielded two fragments, of 12,800 and 5,400 base pairs, respectively; thus, the insert had one *Bam*HI site and no *Hind*III site. pCRI has two *Pst*I sites located about 1,500 base pairs apart¹⁵ (in fact, we have identified a third site, about 100 base pairs removed from one of the previously described sites). The hybrid plasmid has an additional *Pst*I site, introduced by the insert, equidistant from the pre-existing ones. As the distances between the *Hind*III site and the *Pst*I sites of pCRI are known¹⁵, the *Bam*HI site can be located relative to the *Pst*I site of the insert and the *Hind*III site of pCRI (Fig. 2). As reported by Jeffreys and Flavell⁸ and shown in Fig. 3, the order of the restriction sites on the *Kpn*I fragment plus strand (that is, the strand containing the same sequence as the mRNA) is (5')-*Pst*I-*Bam*HI-(3'); therefore, the *Hind*III site is proximal to the 5' end of the globin plus strand.

A partial restriction map of the chromosomal insert was prepared. The restriction sites in the region in and around the globin gene were compared with those of the cloned β -globin cDNA in P β G-1 (ref. 16) (Fig. 3). The maps have been drawn so that the sequences corresponding to the 3'-proximal region of the globin mRNA lie to the right. The single *Bam*HI site is used as reference point for the mapping. There is a clear one-to-one correspondence between several restriction sites mapping between (and including) the *Bam*HI site (position 0) and the *Caul* recognition site at position -185 base pairs. However, the *Eco*RI, *Bsp*I (an isoschizomer of *Hae*III) and *Bgl*II sites, located between 0 and +175 base pairs in the cDNA sequence, are displaced by about 580 base pairs to the right. This displacement delineates the intron previously identified⁸. A further discrepancy between map distances in the chromosomal and cDNA sequences was noted: The *Mbo*II site at -175 base pairs is preceded by a *Bsp*I site at -217 and an *Mbo*II site at -230 base pairs in the cDNA sequence; these distances are increased by about 125 base pairs in the chromosomal sequence, defining a second, smaller intron.

Comparison of the cloned rabbit and mouse β -globin chromosomal DNA fragments by restriction mapping

The mouse β -globin DNA fragment purified from an *Eco*RI digest of chromosomal DNA and cloned in λ gtWES by Tilghman *et al.*⁷ was transposed into the *Eco*RI site of pBR322 by conventional techniques (see Table 1). A partial restriction map of the cloned fragment is compared with that of a mouse β -globin cDNA clone, Z-pCRI/Mc β G-1 (ref. 5) (abbreviated to

Fig. 1 Analysis of partially purified rabbit β -globin DNA. DNA was extracted from livers of inbred Vienna White and Alaska rabbits and digested with *Kpn*I restriction enzyme as described by Jeffreys and Flavell⁹. 3.5 mg of digested DNA were run on a horizontal 0.8% agarose gel (20 × 30 × 1.6 cm) under 50 mM Tris-acetate (pH 7.8), 20 mM sodium acetate and 2 mM EDTA for 70 h at 25 V and 4 °C. The gel was sliced into 1-cm strips, crushed, and the DNA eluted with 150 mM NaCl, 20 mM Tris-HCl (pH 7.5), 2 mM EDTA, and purified on a DEAE-cellulose column as described elsewhere³⁵. Aliquots of 5 μ g of the extracted DNA were run on an analytical 0.8% agarose gel. *a*, The gel was stained with 1 μ g ml⁻¹ ethidium bromide and photographed under short wave UV light. *b*, The DNA from the agarose gel *a* was transferred to a nitrocellulose filter¹¹ and hybridised to P β G-1 DNA labelled by nick translation (specific radioactivity 1–4 × 10⁷ c.p.m. per μ g) as described elsewhere⁹. Lanes 1 and 5, Φ 29 DNA digested with *Eco*RI; lanes 2, 3, 4, fractions from the preparative agarose gel.



M-cG) in Fig. 3. Five restriction sites, from the single *Bam*HI site (taken as reference point) to the *Alu*I site at -165 base pairs, are present at identical distances on both sequences. The *Bsp*I sites located at +48 and +116 base pairs on the cDNA clone are, however, displaced by about 600 base pairs to the right on the chromosomal clone, indicating the intron discovered by Tilghman *et al.*². A second intron of about 120 base pairs is revealed by the displacement of both a *Hind*III and a *Bsp*I site, which on the cDNA sequence are located at -325 and -217 base pairs, and on the chromosomal sequence at -440 and -335 base pairs, respectively. An *Mbo*II site is present at -277 base pairs on the chromosomal DNA fragment and is missing on the cDNA; as shown below, this site lies within the small intron. A *Bsp*I site at -258 base pairs on the cDNA is absent on the chromosomal DNA.

The mouse and rabbit chromosomal DNA segments may be compared using as a point of reference the *Bam*HI site, which is present at homologous positions in both globin DNA sequences, namely within the Val-Asp-Pro codons (amino acids 98-100)¹⁷. It is clear that both globin genes contain two introns each, of similar length and in similar positions.

The analysis carried out so far would not reveal additional, very small introns at other locations within the gene, or even large ones at the extremities of the gene.

Comparison of nucleotide sequences of rabbit and mouse β -globin DNA

Nucleotide sequence analysis of cloned rabbit and mouse chromosomal DNA was carried out by the procedure of Maxam and Gilbert¹⁸, from the restriction sites indicated by horizontal arrows in Fig. 3. The determination of the nucleotide sequence of the β -globin cDNA in plasmid Z-pCRI/Mc β G-1 (Table 1) will be described elsewhere. The amino acid sequence encoded by the cloned mouse cDNA (which was prepared from mRNA isolated from Porton Swiss mice) agrees at the critical positions 13, 20 and 139 with the structure given for β^s -globin (an allele of β -major globin) of C57BL mice, as reported by Popp¹⁹ and amended by Gilman²⁰. It should be noted that our finding of an Asn codon at position 72 and a Ser codon at position 80 agrees with Gilman's sequence rather than with that of Popp. Moreover, we conclude that, in contrast to Popp's sequence¹⁷ there is Asp rather than Ser at position 43 and Ser rather than Asp at position 52, in agreement with Popp¹⁹ and with Gilman (see ref. 17).

The chromosomal DNA MbG2 cloned by Tilghman *et al.*² is from the plasmacytoma MOPC-49 of a BALB/c mouse and should therefore code for a globin of the β^d series^{20,21}. The coding sequences, as far as we have determined (unpublished

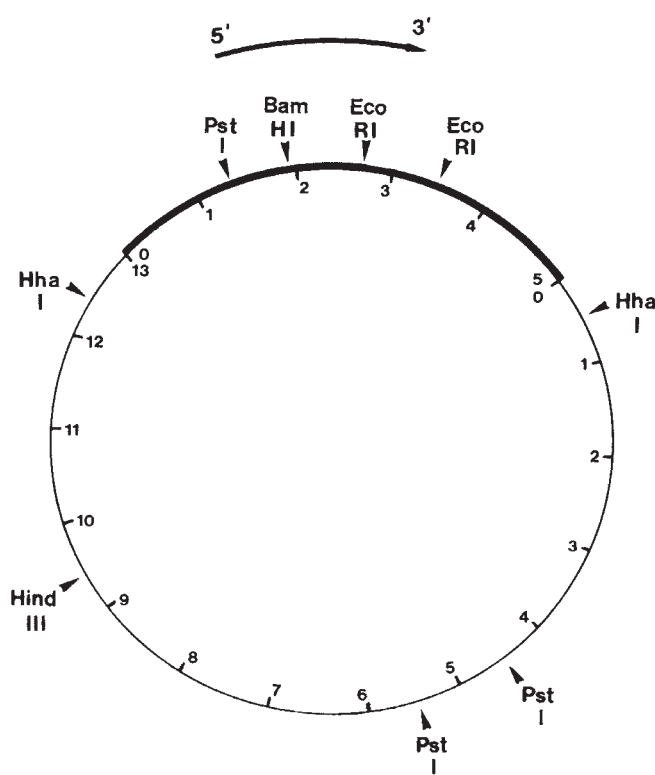


Fig. 2 Orientation of the rabbit β -globin-specific chromosomal DNA in the hybrid plasmid Z-pCRI/Rchr β G-1. The hybrid plasmid was prepared as follows: *Kpn*I-cleaved rabbit DNA enriched for β -globin-specific sequences (the fraction analysed in lane 3 of Fig. 1, about 25 μ g) was elongated with dAMP residues³⁷, annealed to dTMP-elongated, *Eco*RI-cleaved pCRI (ref. 37) (75 μ g) and transfected into *E. coli* χ 1776 by a modification of the procedure of R. Curtiss, III (ref. 38). *E. coli* χ 1776 was grown at 37 °C in 1% tryptone, 0.1% glucose, 0.1% yeast extract, 0.8% NaCl, 0.03% CaCl₂ and 100 μ g ml⁻¹ diaminopimelic acid. At A₆₅₀ = 0.7 (Beckman DB) the cells were chilled, centrifuged, washed successively with 10 mM NaCl and 100 mM CaCl₂, and resuspended in $\frac{1}{25}$ th of the original culture volume of 100 mM CaCl₂. 0.1 ml Ca²⁺-treated cells was added to 0.1 ml of annealed DNA (2 μ g ml⁻¹) in 100 mM NaCl, 100 mM Tris-HCl (pH 7.5), 40 mM CaCl₂ and 40 mM MgCl₂, and incubated for 20 min on ice and for 10 min at 20 °C. Tryptone broth (0.2 ml) was added and the transfected bacteria were plated on agar containing 25 μ g ml⁻¹ kanamycin sulphate, 10 μ g ml⁻¹ nalidixic acid and 100 μ g ml⁻¹ diaminopimelic acid. About 10,000 colonies per μ g of elongated rabbit DNA were found. To transfer the colonies to membrane filters, dry, prewashed Millipore filters were put on top of the colonies, carefully removed and incubated (colony-bearing side up) on fresh agar plates. The membranes were placed on filter paper (Whatman 3MM), soaked with 0.1 M HCl for 10 min and further processed by the method of Grunstein and Hogness¹³. For hybridisation, the filters were preincubated for 4-6 h at 37 °C in 50% formamide containing 0.65 M NaCl, 100 μ g ml⁻¹ denatured salmon sperm DNA, 0.02% polyvinylpyrrolidone, 0.02% Ficoll (Pharmacia) and 0.02% bovine serum albumin³⁹. The filters were then individually hybridised for 15 h at 37 °C under paraffin oil in about 1 ml of the same solution containing 1-3 $\times$ 10⁵ c.p.m. of the 333-base pair *Hae*III globin-specific fragment of p β G, labelled with ³²P-nucleotides by nick translation to a specific activity of 0.5-2.5 $\times$ 10⁷ c.p.m. per μ g, as described elsewhere⁹. The filters were successively washed in chloroform, 50% formamide containing 5 $\times$ SSC, 50% formamide containing 1 $\times$ SSC (twice), and 0.3 $\times$ SSC at room temperature. The dry filters were autoradiographed for several days at -70 °C with an Ilford Fast Tungstate intensifying screen⁴⁰. One positive colony was identified among about 60,000 tested. After recloning the bacteria, plasmid DNA was purified by a modification of the method of Currier and Nester⁴¹. To reclone the DNA, purified plasmid was retransfected into *E. coli* χ 1776 at a low DNA-to-cell ratio. A β -globin-positive clone, *E. coli* χ 1776 (Z-pCRI/Rchr β G-1), was picked and used for all further work. All work involving hybrid plasmids containing chromosomal DNA was carried out in P3 containment conditions using the EK-2 vector *E. coli* χ 1776 (ref. 38). The locations of the *Bam*HI and *Pst*I restriction sites of the insert were deduced from the analysis of *Bam*HI and *Pst*I digests, as well as *Bam*HI-*Hind*III double digests of the hybrid plasmid, using *Eco*RI-cleaved Φ 29 DNA³⁶ as a marker. The *Eco*RI sites were mapped by labelling the 5' termini of the *Bam*HI-cleaved plasmid with ³²P-phosphate¹⁸, digesting the labelled DNA with *Eco*RI and determining the fragment size following electrophoresis on a 1% agarose gel in 50 mM Tris-acetate (pH 7.8), 20 mM sodium acetate and 2 mM EDTA. The fragments were located after staining with ethidium bromide and autoradiography (data not shown). The locations of the *Hind*III and *Pst*I sites in pCRI are from Armstrong *et al.*¹⁵. Only the two *Hha*I sites closest to the insert are indicated; pCRI, but not the insert, contains many additional *Hha*I sites. As the order of restriction sites on the β -globin chromosomal DNA (plus strand) is 5'-*Pst*I-*Bam*HI-*Eco*RI-3' (see Fig. 3), the orientation of the insert could be deduced. The thick line represents the inserted rabbit DNA *Kpn*I fragment, the thin line the pCRI DNA. '0' indicates the erstwhile *Eco*RI site of pCRI. The numbers indicate the distances in 1,000 base pairs (kb). The arrow indicates the direction of transcription. The beginning and the end of the arrow indicate approximately the start and the end of the mature β -globin mRNA as determined by sequence analysis (data not shown).

results), are those of β^d -major and not of β^d -minor globin at the critical positions 58, 73, 76, 77 and 80, as given by Gilman²⁰. As noted above, the chromosomal DNA lacks the *Bsp*I site corresponding to that present at -258 base pairs on the cDNA sequence, the region coding for Gly 13. As β^d -major differs from β^1 -globin by having Cys (UGPy) rather than Gly (GGPy) in position 13, the absence of the *Bsp*I recognition site (GGCC) on the chromosomal DNA can be accounted for. Further, we have detected a (silent) G $\rightarrow$ A difference between the third position of the Lys 66 codon of the cloned cDNA and the chromosomal β -globin sequences (data not shown).

In the case of the rabbit, the encoded amino acid sequence is that of the β -globin described by Best *et al.*²², both in the cDNA¹⁶ and chromosomal DNA (Fig. 4, data shown only in part).

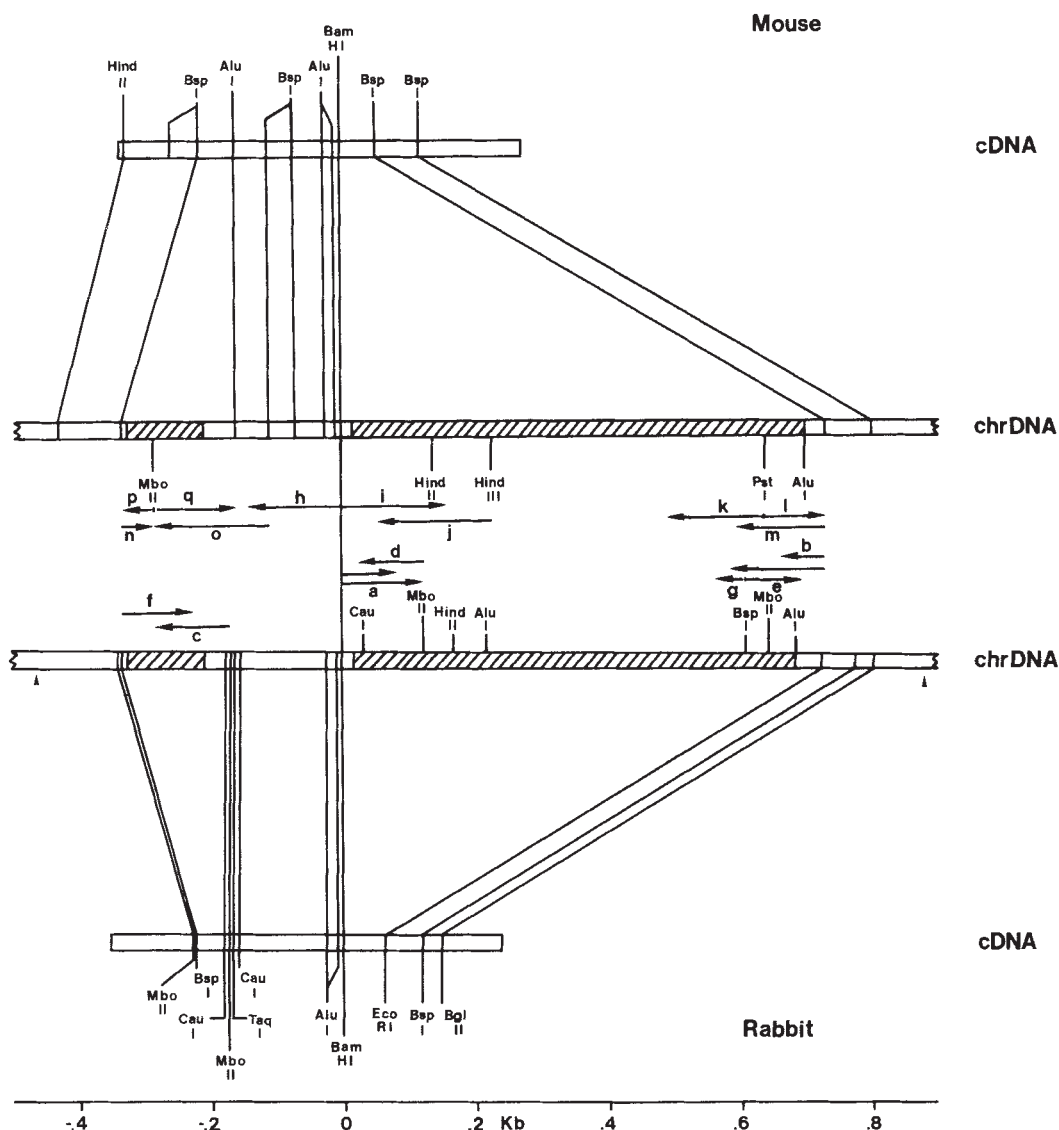
The location of both the small and the large introns relative to the coding sequence is the same in rabbit and mouse. The small introns have been depicted in Fig. 4a as starting after the Arg 30 triplet AGG and terminating with the sequence TPyAGG; both could equally well start with or within the sequence AGG following the Gly 29 triplet, and terminate with TPy, TPyA or TPyAG. A decision between these possibilities can only be

made when the excision sites are established at the level of the mRNA precursor. The large introns (Fig. 4b, c) have been drawn as following the AGG triplet of Arg 104 and terminating with the sequence CAG. In this case the introns might also start within the Arg codon, following the AG, and terminate with the sequence CA.

The small introns differ somewhat in length, that of the rabbit (126 nucleotides) exceeding its counterpart in the mouse (115 nucleotides) by 11 nucleotides. The large introns are not yet completely sequenced and therefore their lengths cannot be compared accurately; however, they seem to be ~580 and 600 base pairs in length in the rabbit and the mouse, respectively.

The large and the small introns show few similarities. One feature they share is the occurrence of strikingly long pyrimidine tracts within the last 30 nucleotides, another is the high T content (31–39% as compared to 23–25% in the coding sequences). As far as the flanking sequences are concerned, the interpretation given in Fig. 4 suggests that both large and small introns are preceded by the sequence CAGG and followed by CTNCTG. However, because of the indeterminacy mentioned above as to the precise position at which the introns start, this feature remains uncertain.

Fig. 3 Comparison of the restriction maps of the cloned β -globin cDNA and the cloned chromosomal DNA segments containing the β -globin genes from rabbit and mouse. The restriction maps of the rabbit and mouse β -globin cDNAs were derived from their nucleotide sequence (ref. 16 and N. Mantei, unpublished results), the rabbit chromosomal β -globin gene was deduced from partial restriction analyses as described by Smith and Birnstiel⁴², using *Bam*HI-cleaved plasmid labelled with ³²P-phosphate at the 5' termini. All the restriction sites were confirmed by sequence analysis (data shown only in part). The restriction map of the mouse chromosomal β -globin DNA was constructed in a similar manner; it agrees essentially with the data reported by Tilghman *et al.*⁷. The map represents only the segment of the cloned DNA about the β -globin-specific sequences. Horizontal arrows indicate the sequenced region discussed in this report. Hatched areas represent introns. The vertical arrows indicate the points corresponding to the ends of the mature rabbit β -globin mRNA as determined by sequence analysis (unpublished results). The distances are given in 1,000-base pair units (kb) as measured from the *Bam*HI site, using Φ X174 replicative form cleaved with *Hae*III as standard. The distance actually measured for the large rabbit intron by agarose gel electrophoresis was 650 base pairs; however, sequence analysis showed that the true value was 580 base pairs. We therefore assume that the length for the large mouse intron (680 base pairs) is also overestimated and use a corrected value of 600 base pairs.



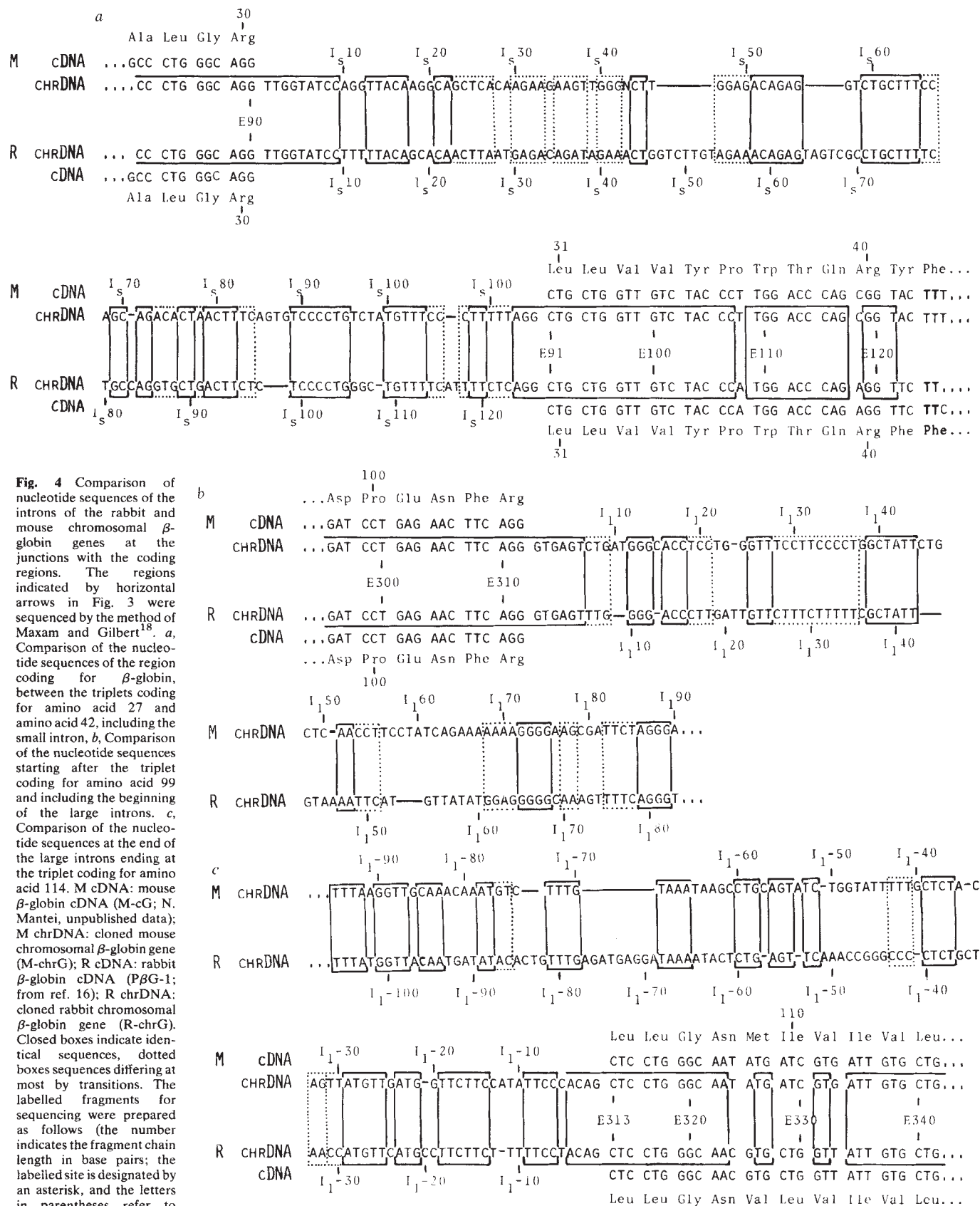


Fig. 4 Comparison of nucleotide sequences of the introns of the rabbit and mouse chromosomal β -globin genes at the junctions with the coding regions. The regions indicated by horizontal arrows in Fig. 3 were sequenced by the method of Maxam and Gilbert¹⁸. **a**, Comparison of the nucleotide sequences of the region coding for β -globin, between the triplets coding for amino acid 27 and amino acid 42, including the small intron. **b**, Comparison of the nucleotide sequences starting after the triplet coding for amino acid 99 and including the beginning of the large introns. **c**, Comparison of the nucleotide sequences at the end of the large introns ending at the triplet coding for amino acid 114. M cDNA: mouse β -globin cDNA (M-cG; N. Mantei, unpublished data); M chrDNA: cloned mouse chromosomal β -globin gene (M-chrG); R cDNA: rabbit β -globin cDNA (PBG-1; from ref. 16); R chrDNA: cloned rabbit chromosomal β -globin gene (R-chrG). Closed boxes indicate identical sequences, dotted boxes sequences differing at most by transitions. The labelled fragments for sequencing were prepared as follows (the number indicates the fragment chain length in base pairs; the labelled site is designated by an asterisk, and the letters in parentheses refer to arrows shown in Fig. 3). **a**, Rabbit chromosomal DNA. (a) Cleavage of plasmid with *Bam*HI, labelling, cleavage with *Eco*RI; isolation of *Bam**-*Eco*-700. (b) Cleavage of plasmid with *Eco*RI, labelling, cleavage with *Bgl*II; isolation of *Eco**-*Bgl*II-1,800. (c) Cleavage of *Hha*-6,200 with *Taq*I, labelling, cleavage with *Hpa*II, isolation of *Taq**-*Hpa*II-1,400. (d) Cleavage of *Hha*-6,200 with *Mbo*II, labelling, cleavage with *Bam*HI, isolation of *Mbo*II*-*Bam*-128. (e-g) Cleavage of *Hha*-6,200 with *Bsp*I, labelling, triple digestion with *Pvu*II, *Bam*HI and *Eco*RI, isolation of *Bsp**-*Eco*-92 (e), *Bsp**-*Bam*-349 (f) and *Bsp**-*Bam*-600 (g). **b**, Mouse chromosomal DNA. (h, i) Cleavage of plasmid with *Bam*HI, labelling, cleavage with *Eco*RI, isolation of *Bam**-*Eco*-2,000 (h) and *Bam**-*Eco*-5,000 (i). (j) Cleavage of plasmid with *Hind*III, labelling, cleavage with *Bam*HI; isolation of *Hind*III*-*Bam*-225. (k, l) Cleavage of plasmid with *Pst*I, labelling, double digestion with *Bam*HI and *Eco*RI, isolation of *Pst**-*Bam*-640 (k) and *Pst**-*Pst*-4,500. The latter was further cleaved with *Bsp*I and *Pst**-*Bsp*-87 (l) isolated. (m) Cleavage of plasmid with *Bsp*I, isolation of *Bsp*-*Bsp*-810, labelling, cleavage with *Bam*HI, isolation of *Bsp**-*Bam*-735. (n, o) Cleavage of plasmid with *Pst*I, *Bam*HI and *Eco*RI, isolation of *Bam*-*Pst*-1,800, cleavage with *Bsp*I, labelling, cleavage with *Mbo*II, isolation of *Bsp**-*Mbo*II-58 (n) and *Bsp**-*Mbo*II-167 (o). (p, q) Isolation of *Pst*-*Bam*HI-1,800 as for (n, o), cleavage with *Mbo*II, labelling, cleavage with *Bsp*I, isolation of *Mbo*II*-*Bsp*-58 (p) and *Mbo*II*-*Bsp*-167 (q).

Figure 4a allows a comparison of the nucleotide sequences of the small introns of the rabbit and the mouse. The sequences were lined up, introducing gaps so as to maximise the homologies. Identical sequences of two or more nucleotides are indicated by a black, continuous frame, and sequences related by nucleotide transitions are framed by dotted lines. The first nine nucleotides from the 5' ends of the introns are identical; elsewhere, several blocks of six to eight nucleotides can be matched. The two introns can be related by 19 point deletions (or insertions), 13 transversions and 26 transitions, for a total of 126 nucleotides. Figure 4b, c shows a comparison of the 5' and 3' terminal regions of the large introns, matched in the same way as described above. The sequences compared comprise about 200 nucleotides; they can be related by 30 point deletions (or insertions), 42 transitions and 29 transversions. As shown in Table 2, the most similar segments of corresponding rabbit and mouse introns are located within 10 nucleotides of the junctions.

Discussion

Is it justified to compare the cloned gene of rabbit β -globin with that of the cloned mouse β^d -major globin? In members of the species *Mus musculus*, adult globin of the β type is coded for by one of at least three different gene arrangements or 'breeding unit alleles': The *Hbb^s* allele leads to the formation of a single type of β -globin, β^s -globin; the *Hbb^d* as well as the *Hbb^p* alleles each consist of two closely linked β -globin genes and are each responsible for the formation of two chains, β^d -major and β^d -minor globin in the first case, and β^p -major and β^p -minor globin in the second²⁰. β -Major globin chains of the p and d type seem to be identical, and differ by three to five amino acids from β^s -globin²⁰. The β -minor globins differ by two to four amino acids; β^s - and β -major on the one hand and the β -minor chains on the other differ by 9–11 amino acids^{20,21}. β^s - and β -major globin are most probably true alleles and represent the ancestral mouse β -globin sequence. The β -minor genes are thought to have arisen by duplication of the β -major gene²¹. In the case of the rabbit, only one adult β -globin type chain²² seems to be formed; at least one additional allelic form differing by a few amino acids seems to exist (Galizzi, as quoted in ref. 17). Evidence for a β -globin mRNA which may be derived from a different β -like locus, has been found in proerythroblasts²³.

We believe that it is justified to compare the cloned rabbit and mouse β -globin genes because both genes encode the major β -globin-like adult chains and are likely to represent the ancestral β -type sequences rather than more recent, duplicated β -globin genes. In any event, the evolutionary distance between mouse and rabbit, as judged by amino acid differences in β -globin (28–29), is substantially greater than that between β -major and β -minor chains, and accordingly, the rabbit β -globin gene is about as similar to the mouse β -major gene (29 amino acid differences in the protein sequence^{17,20,21}; 14 nucleotide differences in the mRNA leader sequence²⁴) as to the mouse β -minor gene (28 amino acid differences, 14 nucleotide differences). Thus, a comparison is justified in any case, as long as the introns in a reduplicated gene also arose by reduplication, that is, are derived from a common ancestral sequence (and were not, for example, inserted into the gene subsequently).

The large and small introns of the rabbit and mouse β -globin genes probably have a common origin because (1) they are located at the same position relative to the coding sequences, (2) they have about the same lengths and (3) they show more sequence similarities than would be expected on a chance basis. To facilitate comparison of sequences, we introduce the 'similarity index' (*SI*), a number which results from the subtraction of the per cent gaps, transitions and transversions required to render the sequences identical, from 100 (Table 2). Thus, the index of identical sequences is 100 and that of two random sequences of equal length and identical base composition is 25. Random sequences of unequal base composition or unequal length may have indices below 25. This index is not entirely satisfactory as it does not take into account that two nucleic acids

having several identical nucleotide positions would be considered more related if these positions were all contiguous rather than scattered over the entire length of the strands. Moreover, the index does not reflect the possibility that different mutations occur with different probabilities; for example, it would seem that transitions occur more frequently than transversions (Table 2), and it seems unreasonable to assign the same probability to the occurrence of one deletion of 10 nucleotides as to that of 10 deletions of one nucleotide. However, there are at present insufficient data to justify the introduction of appropriate weighting factors. Nevertheless, there is some justification for assigning the same value to a point deletion or insertion as to a substitution mutation when comparing sequences which have been aligned by introduction of gaps: when two random nucleotide sequences are compared in this way, the judicious introduction of one gap will on average eliminate about one mismatch.

Using the *SI*, for what it is worth, the β -globin coding sequences as well as the mRNA leader sequence of rabbit and mouse seem closely related (*SI* 81 and 75, respectively). If one considers the so-called silent substitution sites, that is, the codon positions in which nucleotide substitutions do not cause amino acid changes, the *SI* is only 63. The small and large introns have values of 55 and 50, respectively, and are thus as related as the sequences following the termination triplet of the globin gene, which have a value of 53.

Assuming that the introns of rabbit and mouse are derived from common ancestral sequences and that the mutation rate is about the same for all nucleotides, it seems that the strongest constraint (lowest mutation acceptance rate) is on the replacement sites (as defined in ref. 25) within the coding regions, followed by the 5'-terminal leader sequence and the silent substitution sites; the least constraint is on the introns except for about 10 nucleotides at each junction, and the region following the 3' end of the coding region. The high mutation acceptance rate of the introns may also have led to the sequence diversity of the large introns of cloned mouse β -major and β -minor globin DNA detected by Tiemeier *et al.*²⁶ using heteroduplex analyses.

The strong evolutionary divergence suggests that if any function is associated with the introns it does not depend on a unique primary structure of a substantial part of the sequence. Why have homologous introns diverged so widely and yet retained about the same length in mouse and rabbit rather than being largely deleted? Two possibilities must be considered. (1) There is little or no selective advantage in losing part or all of the intron and/or insufficient time has elapsed for such deletions to establish themselves in the population; (2) the presence of an intron with a certain length has a functional significance. What could these functions be? A somewhat remote possibility is that nucleosome or higher order structure of the chromatin depends on some structural feature provided by the introns. It has also been proposed that the presence of introns facilitates recombination and thereby provides selective advantage³.

As regards translation, we may note that one or more stop codons prevent translation through the small and large introns; however, at least short polypeptides could be synthesised from internal intron sequences, from an initiation site within the coding sequence up to a termination codon within the intron, or from a start codon in an intron to a stop codon in the coding sequence. At the level of RNA synthesis and processing, one should note that the mouse β -globin 15S mRNA precursor contains the large⁶ (and most probably also the small) intron and that it is processed to the mature mRNA with a half-life of a few minutes^{4,5}. This processing involves the elimination of the introns by a reaction designated 'splicing'^{27–30} and is thought to involve excision of the intron transcript and rejoining of the RNA ends, in one or several steps³¹. Undoubtedly, the enzyme(s) responsible for such a precise operation have specific substrate requirements. These may be satisfied by specific nucleotide sequences at or about the cleavage sites, and it is striking that the homologies between rabbit and mouse introns are most pronounced around the junctions of the introns with

Table 2 Comparison of nucleotide sequences of cloned chromosomal rabbit and mouse β -globin DNA

Segment	Base composition (%)					Nucleotides compared (R/M)	Gaps	Sequence comparison		Total‡	Similarity index
	A	T	G	C	$\frac{A+T}{G+C}$			Transitions	Transversions		
Coding sequence (including AUG)											
Total	21 20	25 23	31 30	23 27	0.84 (R) 0.75 (M)	441/441	0	49	35	84 (19%)	81
Replacement substitution sites*						441/441	0	16	19	35 (8%) (10%)	92 90
Raw data											
Corrected *											
Silent substitution sites*						132/132	0	33	16	49 (37%) (62%)	63 38
Raw data											
Corrected*											
5' Proximal noncoding mRNA sequence†	32 31	27 27	13 15	28 27	1.4 (R) 1.4 (M)	53/52	1	8	4	13 (25%)	75
3' Proximal noncoding mRNA sequence	26 31	34 32	18 16	22 21	1.5 (R) 1.7 (M)	95/134	41	16	7	64(47%)	53
Small intron											
Total	21 22	34 31	21 24	23 23	1.2 (R) 1.1 (M)	126/115	19	26	13	58 (45%)	55
5' And 3' proximal segments (10 base pairs each)						20/20	0	4	1	5 (25%)	75
Middle segment						106/95	19	23	12	53 (48%)	52
Large intron											
Total available sequences	24 23	39 34	17 22	20 21	1.7 (R) 1.3 (M)	189/185	30	42	29	101 (50%)	50
5' And 3' proximal segments (10 base pairs each)						20/20	0	3	1	4 (20%)	80
Middle segments						169/165	30	40	28	98 (54%)	46

Abbreviations: R, rabbit; M, mouse. Gaps are introduced into the compared sequences to align for maximal homology, as shown in Fig. 4. Gaps are counted by the number of nucleotides they comprise. Similarity index is computed as $100 - (\% \text{ gaps (nucleotides)} \times a + \% \text{ transition} \times b + \% \text{ transversions} \times c)$. The factors a , b and c are taken as 1. % is calculated relative to 'adjusted sequence length'.

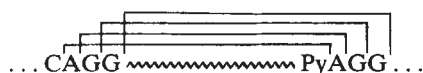
† AUG triplet excluded. The 15 nucleotides at the 5'-terminal region of the mouse β -globin mRNA are from ref. 24. The rabbit sequence is as given in ref. 16.

* See ref. 25. Correction factor is (no. of possible substitutions)/(no. of sites).

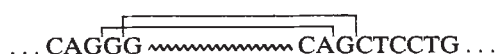
‡ Values in parentheses are % based on 'adjusted sequence length' which is obtained by adding the number of base pairs and the number of unpaired bases (opposite gaps) of the aligned sequences. For example, in Fig. 4a, the adjusted sequence length of the small introns is 130.

the coding regions. In addition, juxtaposition of the cleavage sites could require certain secondary and tertiary structures which may arise by a variety of nucleotide sequences but require a certain length of the intron. Finally, the possibility should be considered that the excised introns themselves fulfil some function, for example, at the regulatory level.

In seeking features common to the large and small introns, we have noted the sequence CAGG at the 5'-junction region of both introns. In the case of the small intron, the 3'-junction region is PyAGG, and the excision of the intron formally seems to be like an internal crossing-over event at any one of four positions:



The situation is rather dissimilar in the case of the large introns where cutting and rejoining does not occur within a region of homology:



Other junctions between introns and their neighbouring regions have been sequenced: six pairs for the ovalbumin gene³², four pairs for immunoglobulin genes (ref. 33 and S. Tonegawa, personal communication) and six pairs in SV40 late and early genes (ref. 34 and S. M. Weissman, personal communication). The fact that the regions at the globin intron junctions are more strictly conserved than in the midpart suggests that they have a

role in splicing. There is a feature common to all intron junctions, namely, a potential cleavage site following either the Pu or the G of a PuG sequence at the junction; the PuG sequence is frequently but by no means always part of the CAGG sequence which has been noted previously³³. There is no obvious feature in common with the intron junctions in yeast tRNAs³¹. There may be several different recognition sequences and perhaps more than one enzyme system for cutting and rejoining; alternatively, or in addition, some as yet unknown secondary or tertiary structure may constitute or contribute to the recognition target.

We thank Dr P. Leder for the mouse β -globin gene hybrid λ gtWES.M β G2, Mr W. Boll for transferring the mouse β -globin DNA segment from λ to pBR322, Mr J. Ecsödi for the preparation of hybrid plasmids, R. Williamson for the preparation of mRNA, and Ms E. de Boer for technical assistance. The work was supported by grants of the Schweizerische Nationalfonds (3.475.75 and 3.114.77) and the Kanton of Zürich to C.W. and by a grant to P. Borst and R.A.F. from the Netherlands Foundation for Chemical Research (SON) with financial aid from the Netherlands Organization for the Advancement of Pure Research (ZWO). A.v.O. was supported by grants from EMBO and ZWO, and J.v.d.B. received grants from EMBO and Koningin Wilhelmina Fonds.

Received 10 August; accepted 29 September 1978.

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letters to nature

An unusual emission-line star/X-ray source/radio star, possibly associated with an SNR

A STAR with an unusual emission-line spectrum is described here which is associated with a variable point radio source, and possibly also with a variable X-ray source and the radio supernova remnant (SNR) W50 (G39.7–2.0). The radio, X-ray and optical properties of the star seem to be similar to those of the radio/X-ray/optical star Circinus X-1, tentatively associated with the supernova remnant G321.9–0.3. Such objects may represent an identifiable subclass of stellar remnants for supernovae.

A point radio source lies near the centre of the old supernova remnant W50 (ref. 1). (The best available position for the point source, from the Cambridge 5 km radiotelescope, is given (data from Mullard Radio Astronomy Observatory, personal communication) as RA 19 h 09 min 21.273 s, dec +04° 53' 53.1" (1950).) The radio source flares at high frequencies, but is relatively quiescent at low frequencies². It lies within the positional error box of the X-ray source³ A1909+04, which is of area 0.06 deg². Although the X-ray source seems to be associated with the SNR W50, it is unresolved and variable (F. D. Seward, personal communication) and hence is not to be identified with emission from the hot, shock-heated interstellar material of the SNR.

The optical counterpart of the radio source was discovered with the 3.9 m Anglo-Australian telescope. Only one star brighter than magnitude 20 lies within 30 arcs of the radio position. This star, magnitude $m_v = 14.2$, is at RA 19 h 09 min 21.29 s, dec +04° 53' 54.8" (1950), standard error 0.3 arc s. A spectrum (obtained with the image dissector scanner, at a dispersion of 100 Å mm⁻¹) is richer in emission lines than that for any other compact X-ray object. It shows a red continuum dominated by broad strong emission lines, including the Balmer series (H α , H β , H γ , H δ), lines from neutral helium, and higher excitation features from ionised helium (He II) and ions of carbon and nitrogen. The higher excitation spectrum is variable; details will be given elsewhere. We note only that the He II 4,686 Å line and 4,640 Å C III, N III, O II complex are regarded as a signature for the optical identification of X-ray sources⁴. The red continuum ($B - V \sim 2.2$ mag) shows no strong absorption lines except for interstellar features such as the diffuse

interstellar lines at 6,284 and 5,780 Å, at a strength correlated with strong interstellar reddening ($E(B - V) \sim 2.4$). Thus the star is intrinsically blue but highly absorbed.

The star shows a striking similarity to Cir X-1, which because of a range of interesting associations and variabilities had previously seemed to be unique. The discovery of another object of the same apparent class suggests that the supposed uniqueness of Cir X-1 merely resulted from its having been studied in greater detail than several other objects with apparently similar properties, in particular A1909+0453. Both Cir X-1 (refs 5, 6) and A1909+0453 are associated with flaring radio and variable X-ray sources. Unlike Cir X-1 (period 16.6 d), A1909+04 is not yet known to be periodic, although clearly variable. Cir X-1 is a more highly reddened star than A1909+04 and its spectrum⁷, detectable redward of about 5,500 Å, is dominated by an intense broad H α line and the same three emission lines from neutral helium which are strong in spectra of A1909+04, namely those at 5,876, 6,678, and 7,065 Å. If the star which we have found associated with A1909+04 were reddened by the same amount as Cir X-1, its red spectrum would appear on at least some occasions to be very similar (Fig. 1).

Finally there is the provocative coincidence that both are associated with SNRs; Cir X-1 with G321.9–0.3, and A1909+04 with G39.7–2.0. The line-of-sight coincidence is in itself suggestive. The number of known early-type emission-line stars would allow a chance positional coincidence with an extended SNR with a probability of ~ 1 in 10. However, there is a much lower probability ($\sim 10^{-4}$) of the chance association of two SNRs with emission-line stars which are X-ray and radio variables, closely resembling each other but showing considerable differences from other ordinary emission-line stars (Be stars, Of stars, WR stars and so on). The spatial coincidence is, moreover, maintained (to some degree) along the line of sight. The distance of Cir X-1 (from 21 cm neutral hydrogen absorption seen against a radio flare⁸) is 8–16 kpc, consistent with the distance⁹ to the SNR G321.9–0.3. On the assumption that A1909+04 is of the same absolute magnitude as Cir X-1, the lower reddening and brighter apparent magnitude place it five times closer than Cir X-1 (ref. 7), that is ~ 1.6 – 3.2 kpc. The distance to W50 is 3.3 kpc (J. L. Caswell, personal communication), consistent to within the uncertainty of the distance estimates, particularly considering the uncertainty of the interstellar absorption.

Cir X-1 has been modelled as a binary system with an eccentric orbit^{10–12} in which a compact X-ray emitter is orbiting

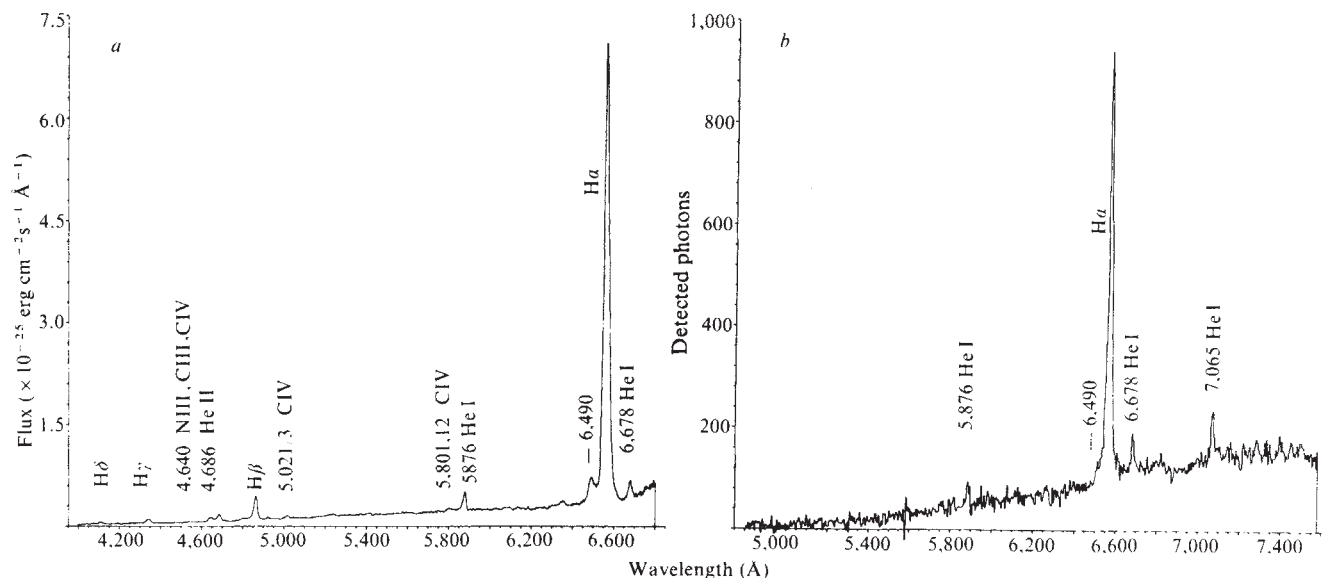


Fig. 1 *a*, Spectrum of A1909+04 on 16 July 1978. Balmer lines of hydrogen and neutral helium lines are most prominent. $\lambda 4686$ He II and the $\lambda 4640$ complex are clear. Possible identifications with CIV lines are indicated. The line at $\lambda 6490 \pm 10$ Å could be the same line identified in 3U1728-24 as $\lambda 6516$ Fe II (ref. 16) or $\lambda 6491.3$ Fe II seen in some nova-like stars¹⁷. *b*, Spectrum of Cir X-1 made on 26 April 1977 with the image photon counting system at a dispersion of 130 Å mm^{-1} , showing He I lines and blueward wing to H α , possibly the same line as in A1909+04.

a massive early-type star. It has been suggested^{10,12} that it is a runaway object from an SNR (G321.9-0.3). We make the same suggestions for A1909+04.

The Molonglo-Parkes survey of SNRs⁹ revealed a statistical excess of point radio sources within or close to the periphery of SNRs. Apart from the Crab and Vela pulsars, none has been identified as a pulsar within the detection limits of existing surveys, but two have been identified optically with unusual emission-line objects and with X-ray variables. This suggests that such objects represent an identifiable sub-class of supernova stellar remnants.

Note added in proof: Seaquist *et al.*¹⁵ note that our optical candidate for A1909+04 is no. 433 in the catalogue of Stephenson and Sanduleak¹⁶.

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Received 9 August; accepted 18 September 1978.

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Submicrosecond time resolution observations of PSR0950+08

PULSARS were discovered by Hewish *et al.*¹ using an instrument with higher time resolution than was conventionally used in radioastronomy. From the short time duration pulses they detected they placed an upper limit of 4.8×10^3 km on the size of the objects, assuming that a radiating region producing a pulse of duration t cannot be larger than ct , the distance light can travel during the pulse. Other observers²⁻⁵ have since reduced this size limit with improved time resolution, with the objective of determining the size of the fundamental radiating elements. We report here our observations of isolated unresolved $0.8 \mu\text{s}$ micropulses, which, if the conventional assumption is made, result from emitting volumes less than 250 m in extent. We also show that even with our high time resolution the observations are consistent with Rickett's amplitude modulated noise model⁶ for pulsar emission, where all of the observed intensity fluctuations can be represented as the modulation of a more fundamental gaussian noise process.

Left-hand circularly polarised signals from PSR0950+08 were observed on 15 May 1976 with the Arecibo 350-m telescope using a 1.25 MHz receiver bandwidth centred at 430 MHz. The i.f. signal was mixed to baseband, converted to 6-bit digital samples, and stored on digital magnetic tape. 32,768 complex samples were recorded in a 26.2 ms window centred on the main pulse for each pulse period. Interstellar dispersion distortion was removed by computer software processing using the technique described by Hankins³, and Hankins and Rickett⁷. In Fig. 1*a* a sequence of consecutive pulses is plotted with the average profile reproduced in the background of Fig. 1*b*. As the intensity pattern from any one pulse is generally very unlike the next, the average profile represents a probability distribution function of pulse intensity with longitude.

The individual pulses can be put into three categories according to their intensity structure: quasi-periodic, noisy and intermittent. From visual inspection of 250 individual pulse records processed in the same way as those in Fig. 1, the quasi-periodic

micropulse periods range from about 0.15 to 1.1 ms with the most frequent periods about 0.4 and 0.6 ms. For two of the pulses the period decreases smoothly over 10° of pulsar longitude from about 0.95 to about 0.44 ms. The noisy pulses are typified by rapidly varying but continuous emission over a substantial fraction of the average profile. Most pulses are of the intermittent type which have bursts of strong emission interspersed with sections of longitude where the detected signal returns abruptly to the system noise level. They contain isolated micropulses ranging in duration down to the $0.8 \mu\text{s}$ resolution limit of the equipment. The micropulse in Fig. 2 is typical of the 20 unresolved micropulses found by examining the 250 individual pulses plotted with the full resolution. As the square-law detection of the signal is done in a computer after software dispersion removal processing, the detector output zero level is

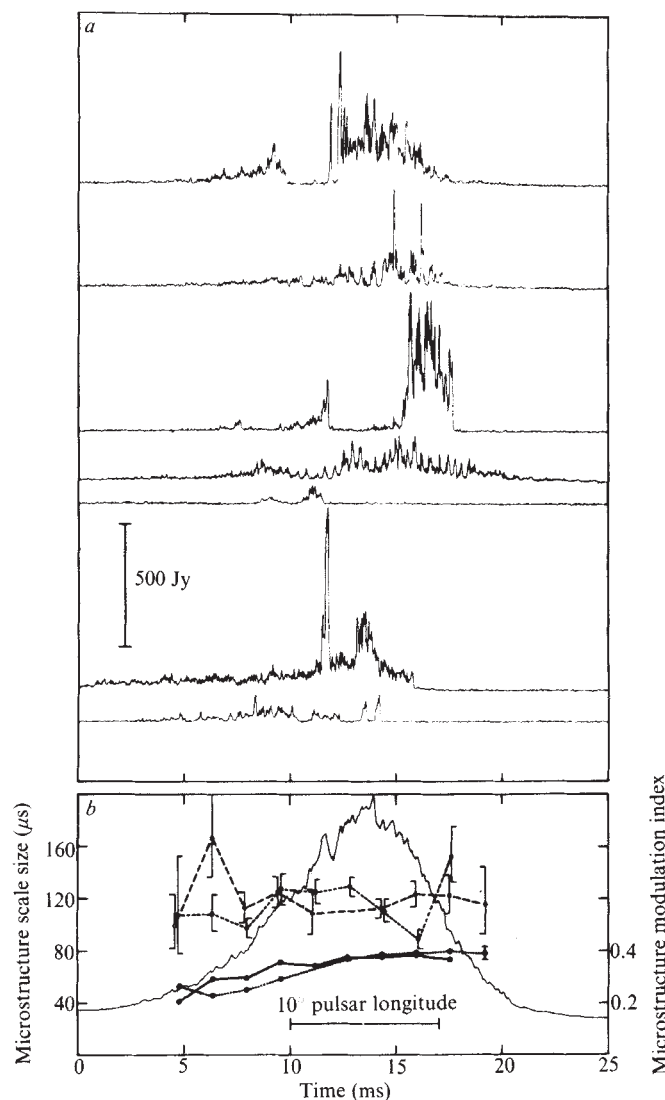


Fig. 1 *a*, A sequence of pulses from PSR0950+08 is shown smoothed up to $16 \mu\text{s}$ resolution. Time increases to the right and upwards. The fourth pulse is of the quasi-periodic type, and the others are of the intermittent type. *b*, The average profile of 250 pulses is shown with the same time scale as the individual pulses. Superimposed upon the average profile are the microstructure scale size, τ_μ , and the microstructure modulation index, m_μ , for two contiguous but independent sequences of pulses. The dashed lines connect measurements of the microstructure scale size; the solid and dotted lines connect the measurements of microstructure modulation index. The error bars indicate $\pm 1\sigma$ limits. Estimation error for the modulation index measurements are smaller than the plotted points.

known exactly, and provides the baseline of Fig. 2. The system noise temperature of 140 K combined with a telescope sensitivity of 14 K Jy^{-1} gives a noise level of about 10 Jy which makes a negligible contribution to the micropulse in Fig. 2. Many resolved micropulses were stronger than the one in Fig. 2. No unresolved isolated micropulses were counted unless they were more than 10 times the system noise level.

A pulsar radio signal $x(t)$ can be represented by a wideband gaussian noise process $n(t)$ modulated by a more slowly varying function $a(t)$ which contains the average pulse, subpulse, and microstructure fluctuations. This model, called the amplitude modulated noise (AMN) model by Rickett⁶, implies that the pulsar signal is of the same character as a thermal radio source except that its flux is rapidly variable. From the high equivalent brightness temperatures measured, the pulsar is clearly non-thermal, but if the pulsar signal has characteristics which are fundamentally different from those of thermal radio sources then one would expect the deviations to be manifested in the signal statistics as departures from the statistics predicted by the AMN model. Rickett⁶ has shown that the intensity autocorrelation function of this amplitude modulated noise signal observed in a receiver bandwidth B is given by $R_I(\tau) = R_a^2(\tau)(1 + \delta(\tau))/2$ where $\delta(\tau)$ is the 'zero-lag spike' corresponding to the autocorrelation function (ACF) of the detected noise process $|n(t)|^2$ after it has passed through the receiver. $\delta(\tau)$ is equivalent to the ACF of the receiver impulse response whose width is B^{-1} . Likewise $R_a^2(\tau)$ is the ACF of $|a(t)|^2$. If the AMN model is valid, $R_I(\tau_1) = 0.5$ where τ_1 is the first lag of the ACF greater than the width of $\delta(\tau)$. A diagram of the expected form of the ACF is shown inset in Fig. 3. The first tests of the AMN model by Cordes⁷ using data from PSR2016+16 showed no departure from the AMN predictions. Our data, which has time resolution a factor of 10 better, also confirms the model. Cordes and Hankins⁹ have also tested and confirmed the AMN model using a similar analysis of the radio frequency structure of micropulses.

Autocorrelation analysis of our data shows a distinct microstructure feature^{4,7,10} with a scale size τ_μ which we have defined by the intersection of two straight lines obtained from a least squares fit over the micropulse and subpulse regions. There is no *a priori* reason for using straight-line fits rather than gaussians or some other function, but for this pulsar straight lines are adequate for characterising the microstructure feature. The microstructure modulation index¹¹, m_μ , is a measure of the relative strength of the microstructure component of $a(t)$ and is given by

$$m_\mu = \left[\frac{R_I(\tau_1)}{R_I(\tau_\mu)} - 1 \right]^{1/2}$$

Figure 3 shows the cumulative ACF of the main pulse. It is the sum of the individual pulse ACFs corrected for the off-pulse noise contribution and normalised so that the 'zero-lag spike' has unity total height. Then, as predicted by the AMN model, the first non-zero lag of the ACF is very close to 0.5. The main pulse then was divided into 10 longitude sectors of 2.3° (1.6 ms) each for ACF analysis. The microstructure scale size τ_μ and microstructure modulation index m_μ were computed for each sector. As shown in Fig. 1*b* there is no consistent variation in τ_μ with longitude, although we found a second breakpoint in the ACFs at $40 \mu\text{s}$ in several longitude sectors. These shorter features occurred only in the first or second half of a 3-min run, but never in both. The existence of the ACF microstructure feature does not imply that microstructure has a unique duration. There can be a wide range of microstructure widths, but micropulses with a significant portion of the total pulsed energy must have a preferred range of duration up to the breakpoint in the ACF. In fact, because the cumulative ACF is weighted by the square of intensity of individual pulses, a few very strong micropulses of $40 \mu\text{s}$ duration could dominate the ACF and produce a non-stationary breakpoint in the ACF. The microstructure modulation index rises from 0.2 at the leading edge up to about 0.37 where it remains for most of the pulse.

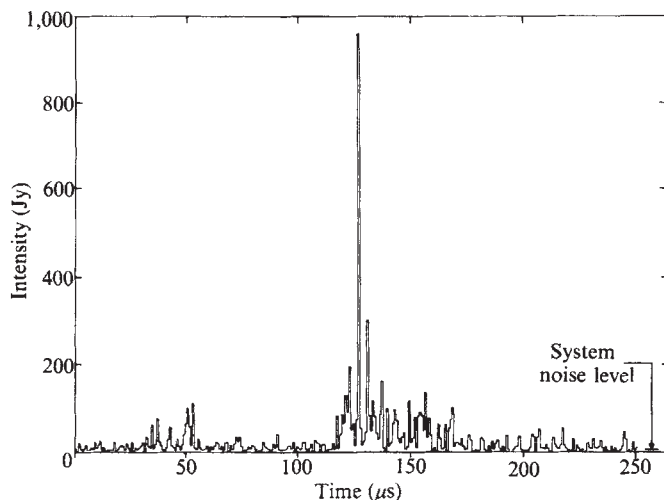


Fig. 2 An unresolved micropulse is plotted with the full $0.8 \mu\text{s}$ resolution. The horizontal base line is the detector zero-output level. Note that the system noise level is negligible.

From the individual pulse plots it is clear that a range of time scales for intensity variations exists, from tens of milliseconds, through the millisecond 'subpulse' range, and now at least to the submicrosecond range where we reach the instrumental resolution limit. If each of these time duration ranges corresponds to a physical scale size, then there are emission regions from 3×10^4 to 3×10^8 cm in extent. From various pulsar polarisation studies it is generally accepted that the average profile time scale is determined by the angular structure of the magnetic polar cap. If instead of attributing the microstructure to temporal modulation we assume that all of the observed time scales result from angular beams of radiation swept past the observer by the pulsar rotation about its axis, then the angular sizes range from 4 arc s to 11° of pulsar longitude assuming that the radiation comes from the pulsar rotational equator. Based on the first assumption, that the shortest emission results from temporal modulation of a source of 3×10^4 cm in extent, the equivalent black-

body brightness temperature of the micropulse in Fig. 2 is 3×10^{31} K requiring a coherent emission mechanism but the energy densities obtained depend strongly upon the exact geometry of the emission region.

In the shot noise model developed by Cordes¹¹, the pulsar radio emission is produced by coherent bunches of relativistic particles travelling outwards along the curved field lines from the magnetic polar cap. Each bunch produces a single pulse by curvature radiation, whose bandwidth is the same as the time average bandwidth of pulsars, that is >1 GHz, and whose time duration is the reciprocal of the bandwidth, or <1 ns. An ensemble of a large number of bunches radiating independently at random times produces by the central limit theorem a white gaussian noise process. The micropulses we have observed must be the envelope or the modulation function $a(t)$ of the noise process $n(t)$. As we find $R_I(\tau_1) = 0.5$ for our data, the AMN model is valid, and we can conclude that in any one time interval $\tau_1 \geq 0.8 \mu\text{s}$ there must be a large number of single shot pulses contributing to $n(t)$. Because we observe occasional unresolved micropulses with our $0.8 \mu\text{s}$ resolution, if we can make the conventional assumption that the physical size corresponds to the light time of micropulse duration, there may be groups or 'clouds' of radiating bunches as small as 2.5×10^4 cm in extent, certainly the smallest distinguishable radiating entities observed outside the Solar System. We should expect departures from the AMN model statistics only if we could improve the time resolution to the point where there are ~ 1 bunches actively radiating during the resolution interval τ_1 . As Cordes has pointed out¹¹, the severe limitations imposed by the required wide receiver bandwidths, plus interstellar dispersion and scattering make it difficult to resolve the individual shot pulses.

We thank Drs J. M. Cordes and B. J. Rickett for helpful discussions. The Arecibo Observatory is part of the National Astronomy and Ionosphere Center, which is operated by Cornell University under contract with the NSF.

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Received 19 June; accepted 16 August 1978.

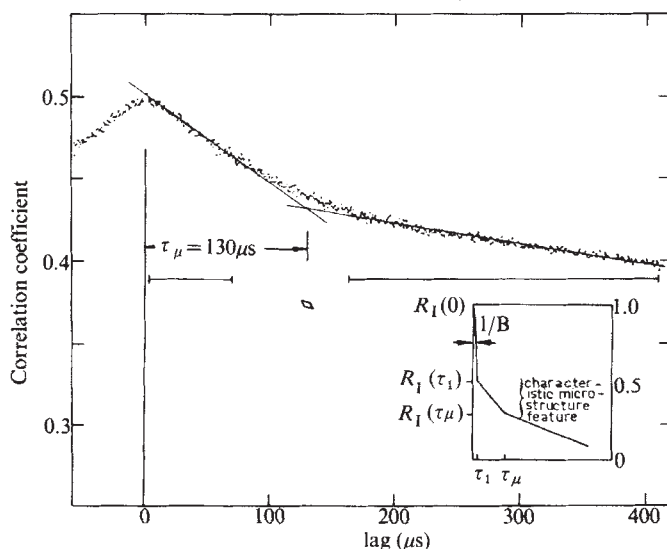
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Transient spectra due to mercury atoms in aqueous solution

THE possibility of obtaining the absorption spectrum of atomic species in the liquid phase was first pointed out by Tyson and West using the electrolysis of various metal ions¹. We provide here new evidence of the existence of mercury atoms in aqueous solution using chemically reducing mercury ion followed by spectroscopic observations.

Figure 1a-d shows the absorption spectra obtained by the chemical reductions of mercurous solutions with various reducing reagents. In spite of the difference in the reducing reagents, the same new absorption band at 254 nm with the doublet structure (251 and 257 nm) appears after the disappearance of

Fig. 3 The intensity autocorrelation function for the main pulse of PSR0950 + 08. The lag resolution is $0.8 \mu\text{s}$. The zero-lag spike has an amplitude of 1.0 and is not shown. The ranges over which least-squares fit lines were computed are designated by the horizontal lines. The 1σ error for the intersection point of the fit lines is an ellipse bounded by the small diamond centred at lag τ_μ but displaced vertically downward for clarity. Inset is a diagram of the expected form of the ACF from the AMN model predictions.



the peak at 238 nm due to Hg(I) ion. This absorption spectrum decays within a few minutes along with the coagulation of mercury to the metal state. Hg(II) ion also renders the same results. Without mercury, the solution does not give any special band in this wavelength region.

The obtained spectra responsible for the reduced state of mercury(I) is close to 253.7 nm of atomic absorption line of mercury in the gas phase, suggesting that it corresponds to the transition from $^1\text{S}_0$ to $^3\text{P}_1$ of Hg(0) . To explain the characteristic doublet structure of the obtained spectra in liquid phase, it is useful to refer to the absorption spectra of Tl^+ , Pb^{2+} , In^+ and Sn^{2+} which have the similar electronic configuration of $d^{10}s^2$. In alkali halide crystals, these elements provide the three absorption bands (A, B and C) at near UV region²⁻⁵, and the doublet structure of A band which corresponds to the transition $^1\text{S}_0-^3\text{P}_1$

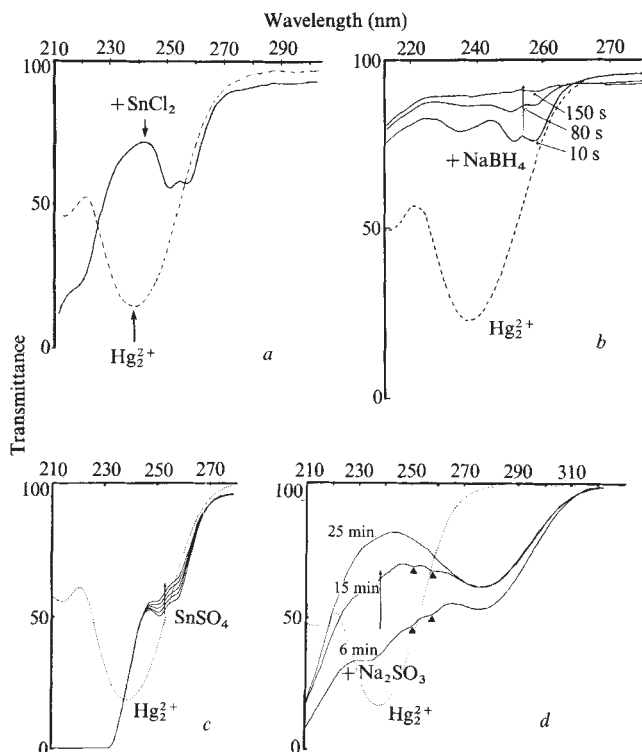


Fig. 1 Absorption spectra of hydrated mercury atoms produced by the chemical reduction of mercurous ions in aqueous solution. Solid and dotted lines are the spectra due to hydrated mercury atoms and mercurous ions in aqueous respectively. Hg_2SO_4 or HgSO_4 was dissolved in deionised and distilled water with 2% sulphuric acid to a concentration of $10 \mu\text{g Hg ml}^{-1}$. Aqueous solutions of 10% stannous chloride or sulphate with 10% sulphuric acid, 10% sodium borohydride, or 10% sodium sulphate was added one or two drops (0.03–0.06 ml) at a time into 2 ml of the aqueous mercurous solution in the quartz cuvette, and the absorption spectra were instantaneously observed using a Hitachi double beam UV-visible spectrometer Model EPS-3.

has been explained by the dynamical Jahn–Teller effect⁶. Although these discussions referred to experiments performed in the solid phase such as the alkali halide crystals, they are still valid in the present liquid phase because the interaction mode of lattice vibration is localised around the central absorber. Then, the dynamical Jahn–Teller effect can be applied to the system of a mercury atom surrounded by coordinating H_2O molecules.

Suppose atomic mercury is coordinated with four equivalent H_2O molecules in the aqueous solution and this coordination belongs to T_d point group. In this case, the Jahn–Teller interaction between the excited state ($^3\text{P}_1$) of mercury and the

vibration of coordinating H_2O can be written, to the second order of the interaction, as

$$H = \begin{bmatrix} -\frac{c^2}{4\Delta}(Q^2 + 3Q_4^2) & -\frac{c}{2}Q_6 + \frac{3c^2}{4\Delta}Q_4Q_5 & -\frac{c}{2}Q_5 + \frac{3c^2}{4\Delta}Q_4Q_6 \\ & -\frac{c^2}{4\Delta}(Q^2 + 3Q_5^2) & -\frac{c}{2}Q_4 + \frac{3c^2}{4\Delta}Q_5Q_6 \\ & & -\frac{c^2}{4\Delta}(Q^2 + 3Q_6^2) \end{bmatrix}$$

where Q_4 , Q_5 , and Q_6 are the trigonal mode of the vibration in T_d group, $Q^2 = Q_4^2 + Q_5^2 + Q_6^2$, Δ the energy difference between A and B bands and c the coupling constant. Other interaction modes, belonging to other symmetries, do not cause any band splitting. The secular equation gives three roots as the eigen states of the above matrix according to the three-fold degeneracy of $^3\text{P}_1$ state. However, from the quadratic terms in the above matrix, the middle and lower components of the above three levels approach each other. Therefore, the absorption band due to the transition ($^1\text{S}_0-^3\text{P}_1$) should have the doublet structure in appearance instead of triplet structure, as already shown by Toyozawa and Inoue⁶, in the case of O_h group. This is consistent with the experimental result.

As only mercury in the reduced state gives the absorption spectrum at 254 nm, and the location of the band is close to the atomic line, and the characteristic doublet structure was successfully explained by the Jahn–Teller effect in the excited state, it can be concluded that the absorption band obtained is due to the hydrated atoms of mercury.

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Received 17 July; accepted 7 September 1978.

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Additive colour properties and colour gamut of cholesteric liquid crystals

LIQUID crystals are organic compounds in a state of matter intermediate between the isotropic liquid and crystalline solid. They are fluid but at the same time they exhibit molecular order. Depending on the ordering one distinguishes the smectic, the nematic and the cholesteric liquid crystals¹ (CLC). In the latter, the rod-like molecules are arranged in layers with their long axes parallel to each other. In each successive layer the direction of the long axis is rotated by an angle of 10–20 arc min; the molecules then form a helical structure. The spacing between layers differing by an angle of 360° is called the pitch, p . Due to the periodicity of molecular orientation, reflections from all layers separated by $p/2$ interfere constructively², if the reflected wavelengths equal the product of the pitch and the refractive indices seen by the wave. This condition is met for a band of wavelength that is relatively narrow and steep and appears as a highly saturated colour^{2,3}. The width of the band equals the product of the pitch and the difference of the two refractive indices of birefringence. Colours produced by such constructive interference, sometimes described as iridescent colours, have also been observed in certain beetles, birds and

butterflies⁴. We report here results of experiments showing that colours of superimposed CLC coatings add like coloured lights and produce a colour gamut greater than that obtained with inks, dyes and pigments.

The unpolarised incident light is separated by the helical structure of CLCs into right-handed and left-handed circularly polarised components². The component that has the same handedness as the CLC used is reflected and the other component is transmitted. The reflected component contains nearly 50% of the energy, as there is little absorption, providing the CLC sample exceeds a thickness of about 10–20 μm . Outside the reflection band there is almost 100% transmission. When two coatings of CLCs with different reflection bands are suitably superimposed, the top coating transmits the wavelengths outside its reflection band and the reflected band from the coating below passes back through the top coating without absorption. Thus the two reflection bands are present and the eye perceives them as the colour of an additive mixture as in the case of lights. This is unlike the colours of inks, dyes and pigments which are determined by the absorption characteristics of the coatings.

The additive properties have been tested experimentally using different samples of CLCs sandwiched between two quartz disks that were spaced by 25- μm nickel foils. The transmittance of the samples was measured in a Cary 14 spectrophotometer.

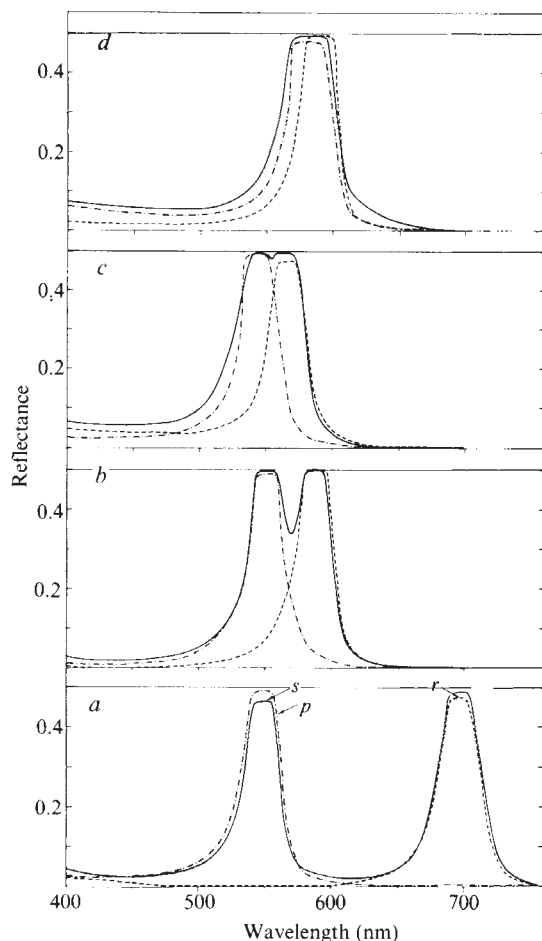


Fig. 1 Reflection spectra of cholesteric liquid crystal samples (mixtures of cholesteryl nonanoate, cholesteryl chloride and cholesteryl oleyl carbonate) centred at different wavelengths, evaluated from transmittance measurements. *a*, Curves *p* and *r* show individual spectra of two different samples; curve *s* shows the spectrum of the two samples superimposed. *b*, *c* and *d*, the spacing between the curves *p* and *r* has been progressively reduced by choosing the mixtures, the reflection spectra of the superimposed samples are centred at the intermediate wavelengths and have an increased width.

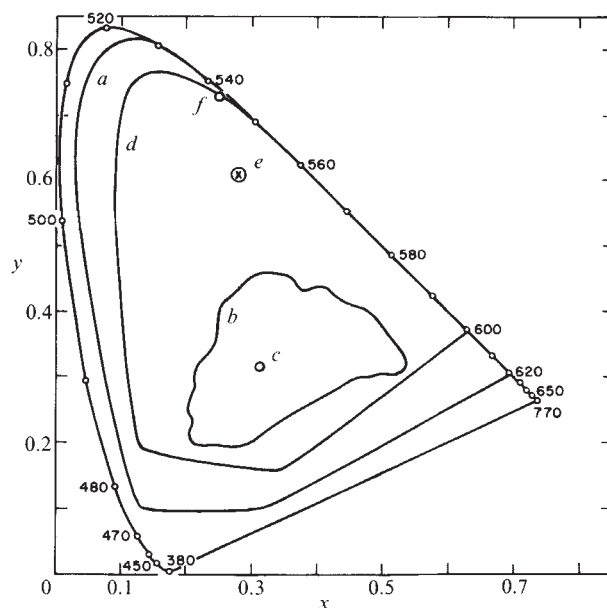


Fig. 2 Chromaticity loci of colours at the luminous reflectance of 0.165 on the basis of the 1931 CIE Standard Observer and source C illuminant. *a*, Optimal colours⁷; *b* Munsell colour standards (evaluated from ref. 8); *c*, location of illuminant C. *d*, Optimum CLC colours; *e*, location of the CLC colour, the spectral reflectance of which is shown in Fig. 1*a*, curve *p*. *f*, Location of the optimum CLC colour having approximately the same centre wavelength and luminous reflectance as curve *p*.

The reflectance spectra were evaluated assuming zero absorption and correcting for surface reflections. A separate experiment confirmed that the sum of the spectral reflectance and transmittance equals unity. Figure 1 shows four situations where, depending on the spacing between the reflection bands of two samples, the spectral reflectance of the superimposed samples appears as two separate bands or, as the spacing decreases, it appears as a single band of almost double width, but the peak spectral reflectance cannot exceed 0.5. By suitable superimposition of individual coatings having reflectances controlled by the thicknesses and centre wavelengths by the CLC mixture it may be possible to synthesise more complicated spectral distributions. Certain aromatic ester derivatives CLCs, such as active amyl alcohol, have a greater difference of the refractive indices⁵ and thus the reflections bands have greater width than those shown in Fig. 1. The above described colour additive properties have been also confirmed using superimposed coatings of encapsulated CLCs.

The colour gamut that is enclosed by the chromaticity loci of colours obtainable with liquid crystals for a given luminous reflectance can be derived as follows. Well-aligned samples of liquid crystals have reflection bands that approach a rectangle with a reflectance of 0.5. Such rectangular spectra will be referred to as those of optimum CLC colours. Except for the peak value, which is 0.5, these spectra are the same as those of the optimum colours, which have a peak value of 1.0 (refs 6, 7). The chromaticity loci of optimum CLC colours can be obtained simply from the loci of optimum colours by reducing by 50% the value of reflectance attached to the latter. The spectra of CLC colours used are not perfect rectangles. To establish the error resulting from the above assumption, the tristimulus values were computed for one CLC colour used, the spectral reflectance of which is shown by the curve *p* in Fig. 1*a*, as well as for an optimum CLC colour having the same centre wavelength and a width that results in the same value of reflectance. The value of luminous reflectance was found to be 0.165. Figure 2 shows the locations of the CLC colour used, the corresponding optimum CLC colour as well as the locus of all optimum CLC colours with the reflectance of 0.165. This is compared with the computed 0.165 locus of Munsell colours⁸ and the 0.165 locus of optimum

colours. It is seen that CLC colours produce a larger colour gamut than the Munsell colours and probably most other colours, as they are much closer to the locus of optimum colours than colours now in use. This comparison, however, should be qualified by the fact that Munsell colours stood up to tests of stability and durability. The CLC colours have yet to be shown to produce equally good coatings. On the other hand, improved alignment of the molecules would shift CLC colours even closer to the optimum locus. Thus the above should be regarded as a discussion of some potential aspects of CLC colours not restricted by practical considerations. Visual inspection of CLC coatings confirm that the colour gamut is greater than that using other colorants. The properties described here may be of interest in colour science, colour displays, art and illustrations of animals which show iridescent colours.

We thank Dr G. Wyszecski for helpful suggestions and R. C. Fink for technical assistance.

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Received 29 June; accepted 15 September 1978.

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Grain-size dependence of cleavage fracture toughness in mild steel

A MODEL has been proposed¹ that relates the cleavage fracture toughness of a mild steel to its yield and fracture stresses through a microstructurally determined characteristic distance. This model quantitatively predicted the experimentally observed temperature dependence of the cleavage toughness in mild steels, and its applicability to low alloy steels has since been demonstrated². In this study, an analytical crack tip stress distribution is combined with this model to provide an expression for the fracture toughness in terms of the microstructurally determined properties. Simply expressed grain size dependences of the yield and fracture stresses are used to predict the grain size dependence of the cleavage fracture toughness of mild steel—it is predicted that the toughness passes through a maximum at grain sizes in the region of 5 μm and that further grain refinement leads to reduced toughness.

Hutchinson³ has presented an asymptotic small scale-yielding solution for the stress distribution at a crack tip in a material obeying the Ramberg-Osgood⁴ stress strain law $\epsilon/\epsilon_y = \sigma/\sigma_y + \alpha(\sigma/\sigma_y)^N$ where ϵ is strain, σ stress, with the subscript y giving yield values, α a numerical constant, and N the work hardening exponent. The maximum principle stress $\sigma_{11}(x)$ at a point x ahead of the tip of a crack of length a is given, in plane strain, by

$$\frac{\sigma_{11}(x)}{\sigma_y} = \beta \left\{ \frac{x}{(K/\sigma_y)^2} \right\}^{-[1/(N+1)]}, \quad \beta = \theta \left(\frac{1-\nu^2}{\alpha I} \right)^{1/(N+1)} \quad (1)$$

where K is the stress intensity at the crack tip and the value of β is available from Hutchinson's solution for θ and I (both of which depend weakly on N). The Ritchie, Knott and Rice model¹ predicts that cleavage fracture will occur when $\sigma_{11}(x)$ first exceeds σ_t , the cleavage fracture stress, over a characteristic distance X_0 . Equation (1) is therefore rearranged to predict the fracture toughness, K_{IC} , as

$$K_{IC} = \beta^{-(N+1)/2} X_0^{1/2} \left(\frac{\sigma_t^{(N+1)/2}}{\sigma_y^{(N-1)/2}} \right) \quad (2)$$

The grain size dependence of the yield stress in mild steel is well established, following the Hall-Petch relationship $\sigma_y = \sigma_0 + k_y d^{-1/2}$ where σ_0 is the internal friction stress, k_y a constant of value 0.74 MPa $\text{m}^{1/2}$ (ref. 5) and d the grain size. It is generally held that the temperature dependence of σ_y arises solely from σ_0 , with the constant k_y being independent of temperature while yielding occurs by slip dislocation motion⁵. Curry and Knott⁶ report a relationship between cleavage fracture stress and grain size for mild steels derived from Smith's model⁷ of cleavage fracture and an experimental grain size:carbide thickness relationship. The fracture stress grain size dependence is shown in Fig. 1, together with the line $\sigma_t = k_t d^{-1/4}$ for a k_t value of 80 MPa $\text{m}^{1/4}$. This empirical expression can be seen to provide a good description of both the derived relationship and the experimental data. It has been shown that the characteristic distance is independent of grain size in mild steel

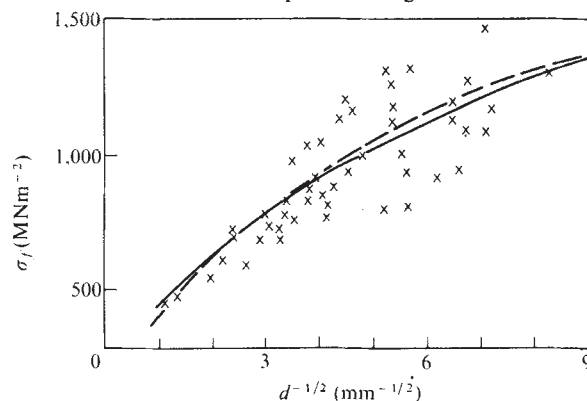


Fig. 1 Variation in cleavage fracture stress, σ_t , with grain size, d , in mild steels. Experimental data of many workers—see ref. 6 for further details. Dashed line, deduced by Curry and Knott⁶; solid line, $\sigma_t = k_t d^{-1/4}$.

for grain sizes of less than 40 μm (ref. 8); the following discussion is restricted to such grain sizes. Note also that the work hardening exponent N may be weakly dependent on grain size (refs 9, 10 present conflicting evidence on the grain size independence of N). As discussed below, the results of this analysis are insensitive to N and so this possible grain size dependence is neglected. If, in the Ramberg Osgood stress-strain law α is set to 1, then N becomes equal to the reciprocal of the conventional Holloman work hardening exponent and hence is of the order of 10. Figure 2 compares real and assumed work hardening behaviour.

The reported grain size dependences for σ_y and σ_t are substituted into equation (2) to predict the cleavage fracture toughness as a function of grain size:

$$K_{IC} = \beta^{-(N+1)/2} X_0^{1/2} \frac{(k_t d^{-1/4})^{(N+1)/2}}{(\sigma_0 + k_y d^{-1/2})^{(N-1)/2}} \quad (3)$$

Taking typical values of $d = 20 \mu\text{m}$, $X_0 = 180 \mu\text{m}$, $\alpha = 1$, $\theta = 2.4$, $I = 4.5$, $\sigma_0 = 220 \text{ MPa}$, and $N = 9$ this predicts K_{IC} to be $\approx 40 \text{ MPa m}^{1/2}$ when a value of $30 \text{ MPa m}^{1/2}$ would be more appropriate⁸. It can be seen that for realistic values of work hardening exponent the predicted toughness is a sensitive function of the singularity amplitude β —a 50% increase in β leads to an eightfold change in toughness prediction for $N = 9$. Since Hutchinson's stress analysis considers elastic strains to be negligible where compared with the plastic strains in the plastic zone, an error in β sufficient to explain the above discrepancy is not improbable. Thus it is unreasonable to use equation (3) to predict the absolute magnitude of fracture toughness as a function of grain size. However, the grain size dependence is independent of β and so should be reliably predicted. Figure 2 shows the product $K_{IC} \beta^{(N+1)/2}$, calculated taking $N = 9$, as a function of grain size for three different σ_0 values corresponding to temperatures at which cleavage is a probable fracture mode.

A maximum in toughness is predicted for all three temperatures, the grain size at which this maximum occurs increasing

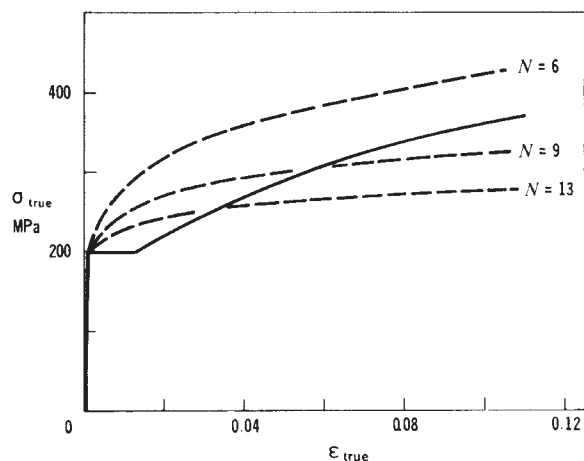


Fig. 2 Comparison between real and assumed stress-strain curves. Experimental data for mild steel after Morrison⁹. Dashed line, from Ramberg-Osgood; solid line, experimental.

with increasing temperature. Differentiation of equation (3) shows this maximum to be located at

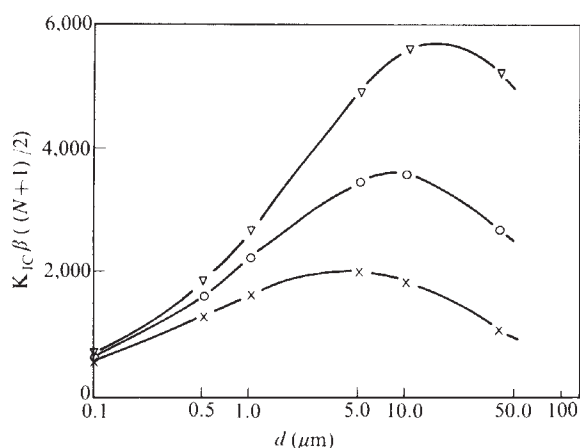
$$d^{-1/2} = \frac{\sigma_0}{k_y} \left(\frac{N+1}{N-3} \right) \quad (4)$$

The temperature dependence of the optimum grain size clearly arises from that of the friction stress, σ_0 . Although the turning point in toughness vanishes for $N \leq 3$, this does not affect the discussion since such a high work hardening rate is not displayed by mild steels. The predicted optimum grain size is not strongly dependent on N for realistic work hardening exponents ($N \approx 10$). Taking $N = 9$, the optimum toughness is given by a grain size of $\approx 3.5 \mu\text{m}$ for $\sigma_0 = 220 \text{ MPa}$ ($T \approx -110^\circ\text{C}$), and for $\sigma_0 = 110 \text{ MPa}$ ($T \approx -50^\circ\text{C}$) the maximum is found at $d \approx 16 \mu\text{m}$.

The prediction of a maximum toughness beyond which further grain refinement leads to a decrease in toughness is at the same time both surprising and rather reassuring. Surprising in that experience has suggested that grain refinement (down to, say, $15 \mu\text{m}$) always produces a tougher as well as a harder steel, and reassuring in that every other hardening process causes a reduction in toughness. This anomalous behaviour arises because in mild steels the cleavage fracture stress is determined primarily by the carbide thickness which, although metallurgically linked to the grain size⁶, is not directly proportional to it. In consequence, the ratio of yield to fracture stress varies with grain size.

Clearly the predictions of this simple analysis must be regarded as speculative. It is based on an approximate stress analysis

Fig. 3 Variation with grain size, d , of predicted relative fracture toughness, $K_{IC} \beta^{(N+1)/2}$ (calculated for $N = 9$), ∇ , $T = -50^\circ\text{C}$; $\sigma_0 = 110 \text{ MPa}$. $\circ$, $T = -80^\circ\text{C}$; $\sigma_0 = 150 \text{ MPa}$. $\times$, $T = -110^\circ\text{C}$; $\sigma_0 = 220 \text{ MPa}$.



and, to some extent, on the extrapolation of existing experimental data. Nevertheless, dimensional considerations show the functional dependence of toughness on grain size to be correct and hence the location of the maximum toughness is unlikely to be significantly in error.

The implications of this study for the production and structural utilisation of mild steels are considerable. First, Fig. 2 shows that the grain size for maximum toughness varies with temperature. Thus it is predicted that an optimum grain size specified for one temperature may maximise toughness at that temperature, whilst giving less than optimum toughness at a different temperature. Second, it is predicted that the optimum grain size lies in the range 3 to $15 \mu\text{m}$ for typical temperatures at which fracture would be expected to occur by cleavage, further grain refinement leading to hardening at the expense of reduced toughness. Controlled-rolled steels are now being produced with grain sizes of the order of $5 \mu\text{m}$ as a possible alternative to alloy steels for structural use. The prediction of the present study is that a mild steel with a grain size of $5 \mu\text{m}$ can be less tough than one of $40 \mu\text{m}$ grain size. If the carbide-grain size and hence the fracture stress-grain size relationship in controlled-rolled steels is the same as that observed in normalised and annealed steels (see ref. 6), then the efficacy of controlled-rolling as a general route to higher toughness steels is called into question (although obviously the present predictions do not apply to controlled-rolled steels alloyed in such a way as to change significantly the carbide particle dispersion, for example giving V_4C_3 instead of Fe_3C and hence changing the fracture stress:grain size relationship.)

Knott¹¹ has drawn attention to the need for an understanding of the micro mechanism of fracture in the design of materials with improved resistance to fast fracture. This study illustrates how quantitative microstructural modelling of failure can be combined with fracture mechanics analyses to make predictions about optimising a steel's toughness. It indicates an obvious need for an experimental investigation of the microstructural dependence of fracture toughness in the new fine grained steels.

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Received 19 July; accepted 19 September 1978.

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Wind shear in a monsoon depression

DEPRESSIONS of the Bay of Bengal are an important component of the south-west monsoon circulation. Moving WNW, they account for much of the rainfall in India. Synoptic conditions favouring formation of monsoon depressions, so far described, are: (1) trailing of the axis of the monsoon trough into the head Bay of Bengal; (2) movement of a low pressure system from the east across Burma; and (3) the building downwards of a midtropospheric cyclonic circulation¹. Studying the structure of monsoon depressions in their later stages of evolution, using principally upper air data of land stations, earlier workers² have invoked upper divergence as a crucial factor for development. Gray³ questions the role of upper divergence in tropical cyclogenesis and emphasises that small vertical wind shear is a primary factor in storm development. The mechanism of initial formation over the Bay, however, has remained unexplored for

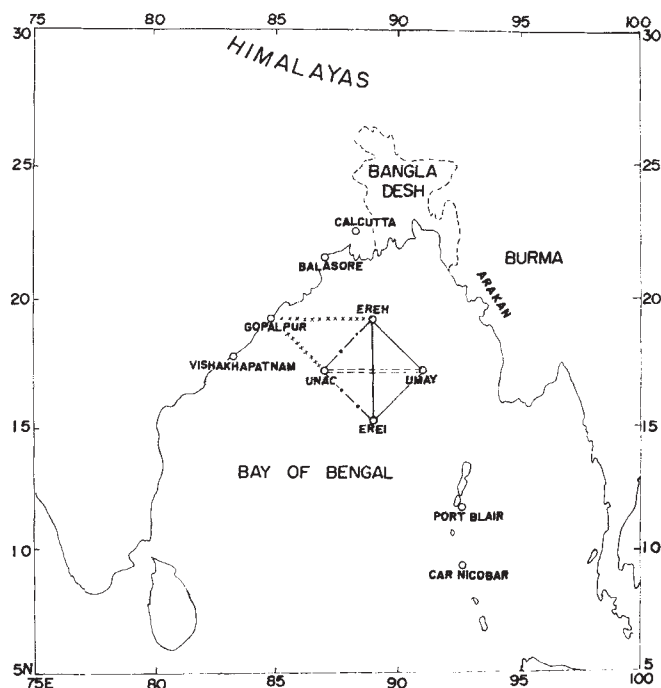


Fig. 1 Locator chart: Soviet ships Ereih, Umay, Ereih and Unac in polygon formation over north Bay of Bengal. Triangles used in computation of vorticity are indicated.

want of adequate data. To gain further insight, a close network of ships and coastal and island stations was organised in the joint Indo-USSR Monsoon-77 Experiment (Fig. 1). Four Soviet research ships in polygon formation recorded for the first time, upperwind observations over the Bay of Bengal. The formation of a depression in the north Bay just to the east of the ships on 19 August and its passage across the polygon the next day was monitored. Sharp decrease in tropospheric vertical wind shear was identified along the coast to start with and subsequently over the north-east of the polygon, before formation of the depression. Marked anticyclonic vorticity first appeared in the upper troposphere and was then totally replaced by cyclonic vorticity of comparable magnitude. This cyclonic vorticity gradually descended to the lower troposphere. The above features,

hitherto undocumented, are described here.

The features in the lower troposphere during the period 12–18 August were typical of a 'break' situation in the south-west monsoon. Chances of monsoon depression formation in the Bay were bleak although the sea surface temperature was of the order of 28.5 °C.

Isolobars and wind changes in the troposphere below 500 mbar suggested that a diffuse perturbation in the westerlies had apparently moved eastwards across north-east India and extreme north Bay between 14 and 16 August. In support, the satellite picture showed on 17 August a well marked north-south band of cloudiness along Bangla Desh–Burma coast, extending up to 17° N. Concurrently, a pressure fall of 3 mbar was registered over the ship area and a trough appeared along the Arakan coast of Burma with the coastal stations it commenced to rain. The polygon area in the Bay was then devoid of clouds (Fig. 2). On 18 August, a further fall of 2.5 mbar occurred in the eastern half of the polygon and a low formed *in situ* with centre at 19.5° N and 92.5° E.

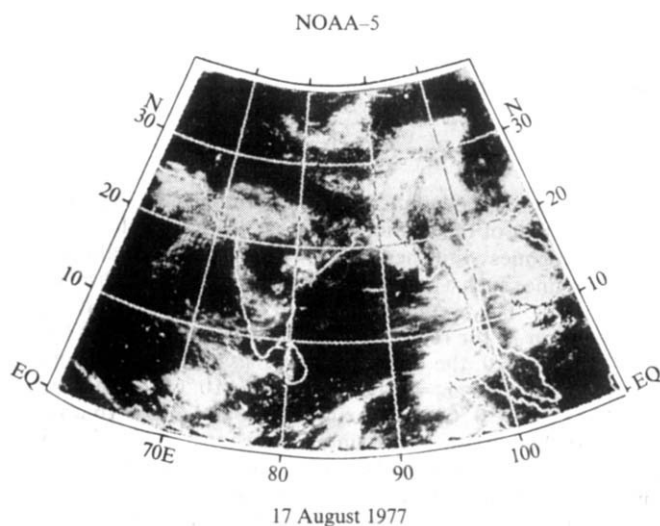


Fig. 2 NOAA-5 visible Satellite pictures 0235 GMT at 100° E on 17 August 1977. Note cloudiness along Bangla Desh, Burma Coast. The polygon area in the Bay is devoid of clouds although pressure fell by 3 mbar.

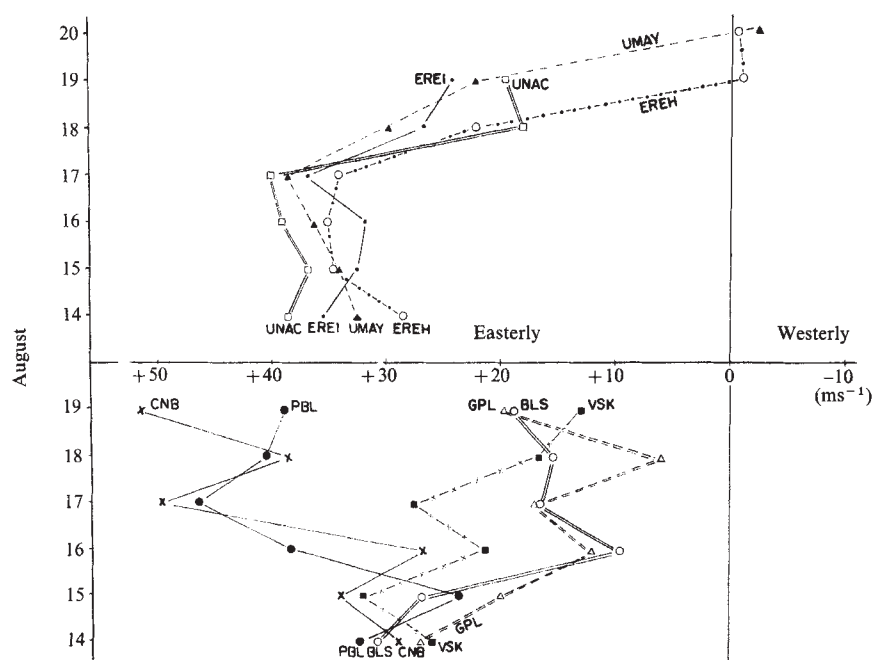


Fig. 3 Variation of zonal vertical wind shear

$$u_{200} - u_{850}$$

m s^{-1} from 14 to 20 August 1977 recorded by the Soviet ships, two island stations and three coastal stations. Easterly zonal wind taken as positive. Coastal stations show decrease in shear earlier. Ships Ereih and Umay register sharp decrease in shear between 17 and 19 August, 1977. Data pertain to 00 GMT; where not available data of 06–12 GMT used.

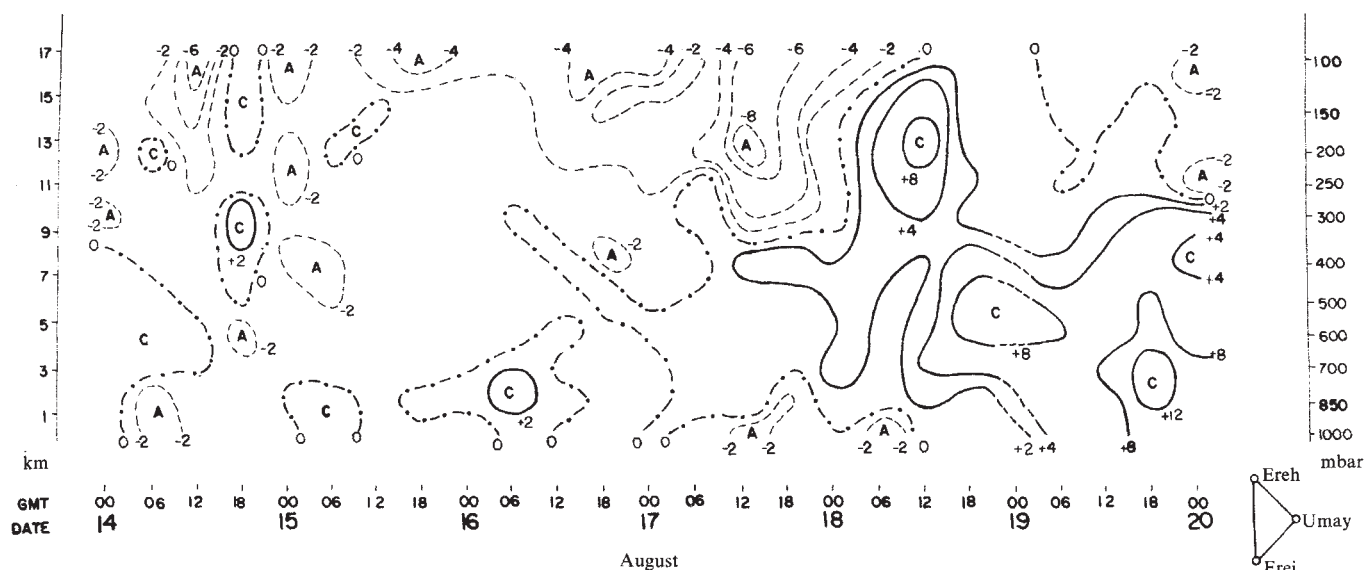


Fig. 4 Daily variation of vertical component of vorticity (10^{-5} s^{-1}) over eastern triangle (Ereh, Umay, Erei) from 14 to 20 August 1977. Large cyclonic vorticity noticed in eastern and southern triangles (Unac, Umay, Erei) to south-west of depression centre.

For convenience, we take easterly wind as positive. The normal vertical wind shear between 200 and 850 mbar in August at 17.5° N is large ($+25 \text{ m s}^{-1}$). Figure 3 shows the daily zonal vertical wind shear recorded by the ships and coastal and island stations. Wind shear at the ships' position in the north Bay of Bengal continuously decreased from 17 to 19 August and even on 20 August at one of them. The same feature is displayed by the coastal stations on one or two days. The largest decrease in wind shear seems to have been confined to a 300 km wide belt from the coast to the two northeastern ships (Ereh and Umay). It is in the eastern half of this region that the incipient disturbance developed into a depression in the 48-h period since shear began to decrease on 17 August. The order of low shear ($\Delta u = 10 \text{ m s}^{-1}$) that occurred at two coastal stations on 16 August was attained only at the two northeastern ships and that too, two to three days later. This suggests that the decrease in wind shear was, perhaps, not caused by the depression itself but occurred independently and well in advance of cyclogenesis.

Vorticity was computed forming triangles of the four ships as well as with the coastal stations. When the depression centre was within the polygon on 20 August, cyclonic vorticity prevailed in the lower troposphere up to 400 mbar, with anticyclonic vorticity aloft (Fig. 4). Taking a total view of vorticity distribution in and around the depression field, the strongest cyclonic vorticity was in the south-west quadrant. The magnitude of the cyclonic and anticyclonic vorticities exceeded the value of the coriolis parameter ($0.7 \times 10^{-4} \text{ s}^{-1}$).

From 14 to 17 August, anticyclonic vorticity in the upper troposphere waxed and waned, while the values in the lower troposphere were negligible and indifferent. On 17th anticyclonic vorticity at 200 mbar was marked and extended right up to the coast. On 18 August, however, the whole atmosphere over the polygon developed cyclonic vorticity, being most marked at 200 mbar level. The low pressure area off the Burma coast was to the north-east of the polygon on 18 August. By 19 August the level of maximum cyclonic vorticity seems to have gradually descended to the lower troposphere with anticyclonic vorticity appearing in the upper troposphere on that day and thereafter.

The sequence of strong anticyclonic and cyclonic vorticity in the upper troposphere has occurred $\sim 300 \text{ km}$ south-west of where the depression centre developed on the morning of 19 August. The vorticity change in the upper troposphere in the

polygon gives:

$$\frac{\partial \zeta}{\partial t} = 0.75 \times 10^{-5} \text{ s}^{-1} \text{ h}^{-1}$$

Compositing the changes with respect to the movement of the low and with a wind velocity of about 40 knots at 200 mbar

$$u \frac{\partial \zeta}{\partial x} = 5 \times 10^{-5} \text{ s}^{-1} \text{ h}^{-1}$$

Decrease of vertical wind shear below 10 m s^{-1} from the normally prevalent large value seems to be a precursor to formation of monsoon depression over the Bay of Bengal. Shear and vorticity changes seem to be confined to a scale of 300 km and time span of 24 h. A polygon layout of $\sim 300 \text{ km}$ side may be just the optimum size for monitoring tropical disturbances. Besides providing significant information for numerical simulation models, the results of this study should be useful in planning the most desirable observational pattern during Monex-79 for further intensive study of the mechanism of monsoon depression formation.

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Received 21 June; accepted 1 September 1978.

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Past atmospheric composition and climate, gas parameters measured on ice cores

VARIATIONS of physical and chemical properties of ice cores have been observed by many authors¹⁻³. These variations could be attributed to temperature effects, different origins of moisture and changes in concentrations and isotopic composition of atmospheric constituents. We report here data from parameters measured in a section of the Camp Century ice core from Greenland. Gas content and composition were measured systematically in a continuous set of samples covering $\sim 2 \text{ yr}$ of

precipitation. The data are compared with measurements of $^{18}\text{O}/^{16}\text{O}$ ratio, electrical conductivity, pH values and crystal size. The aim of the investigation was to determine which parameters show random and which seasonal variations. Special consideration is given to the CO_2 content, which is higher in the gas extracted from natural ice than in the atmosphere⁴ and shows short-term variations which cannot be explained by variations of the atmospheric CO_2 content alone. These probably reflect variations in the meteorological conditions at the time of precipitation. Meteorological conditions may influence other parameters of the ice in a similar way. If such correlations could be found, one might be able to correct the observed CO_2 variations for the meteorological effect and sort out the effect related to changes in the atmospheric CO_2 content. Measurements of the CO_2 content of ice cores of different age could then be used to reconstruct the history of the atmospheric CO_2 content from present to 100,000 yr BP.

Our samples of the Camp Century ice core are taken from a depth of 90.2–90.9 m below surface. Ice at this depth was formed from snow that fell ~180 yr ago. The age of the ice and the age of the air occluded in it are not identical because gases only became occluded at a depth of 70 m. The annual precipitation in Camp Century is ~35 cm water equivalent, corresponding to an ice layer of ~41 cm at the depth of 90 m. Thus, with a sample thickness of 3 cm, about 14 samples per year were studied. Total gas content and composition of N_2 , O_2 , Ar and CO_2 were measured. Parallel samples were used to determine

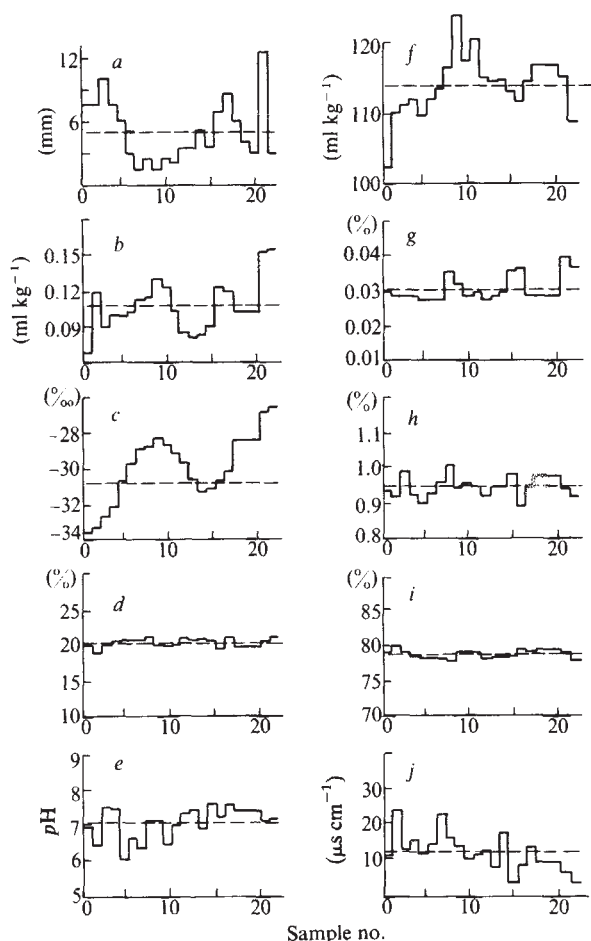


Fig. 1 Time series of physical and chemical properties measured on a section of the Camp Century ice core, Greenland; depth range 90.2 to 90.9 m below surface. The dashed lines give averages for the data series. *a*, Crystal size; *b*, CO_2 content; *c*, $^{18}\text{O}/^{16}\text{O}$ ratio; *d*, O_2 ; *e*, pH; *f*, gas content; *g*, CO_2 1st fraction; *h*, Ar; *i*, N_2 ; *j*, electrical conductivity.

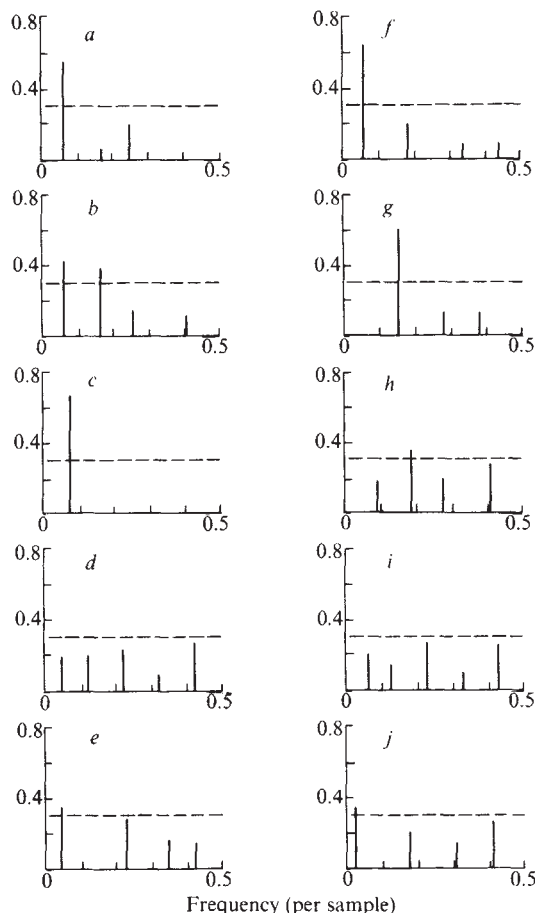


Fig. 2 Weighted frequency spectra after the harmonic analyses according to the maximum entropy method. The amplitudes of the frequency lines are a measure of its contribution to the total variance of the time series. The dashed lines show the levels of significance. *a*–*j* as in Fig. 1.

average crystal diameter and, on the melted samples, electric conductivity, pH value and $^{18}\text{O}/^{16}\text{O}$ ratio.

The experimental procedure for the determination of gas content and composition has been described previously⁴. During each gas extraction the CO_2 content is measured twice. The first analysis is carried out after ~7 min of extraction, that is, as soon as sufficient gas for a measurement is available. This occurs before the ice has completely melted. A second analysis follows after ~7 h of extraction, when all the CO_2 has been extracted. We assume that the first fraction represents the air enclosed in the bubbles. This is supported by detailed studies of a greater number of extraction fractions and of ice cores of different origin.

The average crystal diameter along an axis is estimated using a polarising microscope. Electrical conductivity and pH value are determined when the sample has melted and is in contact with the laboratory atmosphere. The $^{18}\text{O}/^{16}\text{O}$ ratio is determined using a mass-spectrometer. The results are given in Fig. 1. Apparently the $^{18}\text{O}/^{16}\text{O}$ ratio, the crystal size, the total gas content and the total CO_2 content show sympathetic variations, in the case of the crystal size with opposite phase. For the other parameters such a correspondence is not evident. For all possible combinations of two sets of measurements the cross-correlation coefficients have been calculated. Significant coefficients were obtained for the following pairs of sets only:

$^{18}\text{O}/^{16}\text{O}$ ratio,	total CO_2 content	0.646;
CO_2 first fraction,	total CO_2 content	0.586.

The correlation between total CO_2 content and first CO_2 fraction may result from evolution of dissolved CO_2 during the

extraction of the first fraction. Coefficients slightly below the level of significance are obtained for the following pairs of parameters:

Total gas content,	$^{18}\text{O}/^{16}\text{O}$ ratio	0.552
Electrical conductivity,	CO_2 first fraction	-0.498
Electrical conductivity,	pH value	-0.465
Total gas content,	crystal size	-0.438

The level of significance was estimated using the Student's *t* test. The degree of freedom for each set of measurements has been calculated using the auto-correlation function. In Fig. 1 the averages for all the samples are also plotted. Note that compared to the atmospheric composition, the oxygen content is slightly lower and the argon content slightly higher: O_2 , 20.34% (measurement) and 20.95% (atmosphere); Ar, 0.948% (measurement) and 0.934% (atmosphere). Such deviations have already been reported⁵⁻⁷.

To estimate the influence of seasonal variations, all the data were harmonically analysed using the maximum entropy method. The weighted frequency spectral of all 10 sets of measurements are given in Fig. 2. Spectral lines with an amplitude higher than 0.3 are significantly different from random variations (white noise). Table 1 lists dominant frequencies. Column 3 shows the percentage to which the variation of the corresponding frequency is responsible for the total variance of the series. Column 4 shows the corresponding wavelengths in the unit cm of ice. At a depth of 90 m at Camp Century, we expect annual layers with a thickness of 41 cm ice. The $^{18}\text{O}/^{16}\text{O}$ ratio, the total gas content and the crystal diameter are characterised by a dominant frequency, the wavelength of which

Table 1 Dominant frequencies of the harmonic analyses using the maximum entropy method

Parameter	Frequency (sample ⁻¹)	Contribution to the total variance (%)	Layer thickness (cm ice)
$^{18}\text{O}/^{16}\text{O}$	0.072	66.8	41.7
Total gas content	0.060	63.3	50.0
Total CO_2 -content	0.066	42	45.5
	0.171	38.8	17.5
CO_2 1st fraction	0.152	60.4	19.7
Ar	0.190	35.3	15.8
N_2	—	—	—
O_2	—	—	—
Electrical conductivity	0.033	37.3	90.9
pH	0.045	37	66.7
Crystal size	0.064	55.6	46.8

corresponds to an ice layer of 41.7–50 cm thickness. The variation of the total CO_2 content shows two almost equally marked frequencies. The lower frequency corresponds to an ice layer of 45.5 cm thickness. This indicates that meteorological effects showing seasonal variations are partly responsible for the variations observed for each of the four parameters mentioned above. For verification of this hypothesis we plan to measure longer ice cores representing several years. The fact that for the 1.5 yr investigated the annual layer thickness for the four data series are not of equal length indicates that no single meteorological factor is responsible for the variations. If, for example, the maximum of the $^{18}\text{O}/^{16}\text{O}$ ratio represented the summer, that is the warmest precipitation, and if the maximum of the crystal size an autumn event, the difference in the thickness of the annual layer for the two time series could be explained by an irregular distribution of precipitation during the 1.5 yr. The seasonal variation of the crystal size may be due to two fundamentally different processes. Seasonal variation of crystal size in the uppermost snow layer caused by temperature gradients may not be homogenised at a depth of 90 m. Alternatively an indirect seasonal pattern in crystal size may be stabilised as a result of differential recrystallisation velocities in ice at depth, due to the

influence of impurities. The dust content of ice shows seasonal variations³.

The total gas content of ice formed by dry sintering probably depends on crystal size and crystal form at the transition zone firn-ice. The variations in gas content would thus be influenced by seasonal changes in crystal size. Such effects should be considered when interpreting differences between average gas contents of holocene ice and pleistocene ice⁸. The high CO_2 content and its variations could be understood if either during the formation of the snow or during the sintering process of snow to ice the liquid phase were present (freezing of super-cooled droplets or melting at the snow surface in summer). However, as the mean annual temperature at Camp Century is -24°C , the influence of the liquid phase can be neglected. At this temperature sublimation is the dominant mechanism for the formation of snow and the metamorphosis from snow to ice occurs through dry sintering. It is not yet known how the CO_2 is enclosed in ice during these processes. Measurements have shown that most of the CO_2 is already present in freshly fallen snow. We assume that the CO_2 content of snow depends among other parameters on the CO_2 partial pressure and on the temperature during snow formation.

The frequency spectra of Ar, O_2 , N_2 , pH and electrical conductivity measurement sets indicate random variation. For the first fraction of the CO_2 content the seasonal variations are missing, in contrast to the behaviour of the total CO_2 content. This confirms that this part of the CO_2 is enclosed in ice in a different way from in snow. The CO_2 content of the gases of the first fraction varies between 0.027 and 0.039%. The lower values ($<0.03\%$) are within the range of the estimated pre-industrial atmospheric CO_2 content of 290 p.p.m. (ref. 9) or lower (260–280 p.p.m.) if there was a considerable CO_2 release by forest burning during the past hundred years¹⁰. This agreement may be accidental and we would like to emphasise the preliminary character of these results. For the determination of the pre-industrial atmospheric CO_2 content, additional measurements on firn and ice cores from areas with different meteorological conditions, representing the precipitation of several years, are needed. In particular, we shall measure the CO_2 content of the bubbles by grinding the ice at -20°C (instead of melting it) and by extracting the gases.

We hope to show with this short series of measurements that systematic studies of a series of parameters in ice core may yield information on the history of the atmospheric CO_2 content. The answer, however, will not be straightforward but implies understanding of the complex processes during snow formation and metamorphosis of snow and ice.

We thank professor Dr C. C. Langway, State University of New York at Buffalo, Amherst, for providing the Camp Century ice core samples and for valuable discussions. We thank A. Neftel for making the statistical analyses. This work was supported by the Swiss NSF.

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Received 8 August; accepted 7 September 1978.

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Dating sediments by a K-Ar method

ATTEMPTS to date sedimentary rocks by isotopic methods have produced controversial results. That sedimentary rocks of known stratigraphic age have not been dated with any great success is considered to be one of the major disadvantages of the K-Ar method¹. Previous attempts have been unsuccessful^{2,3} because sediments contain argon which had been inherited from previous orogenic K-Ar events before weathering, transportation and deposition. This means that the time of sedimentation cannot usually be obtained by the standard calculation. A new method of dating sediments proposed here attempts to overcome this problem by using data from size-fractionated argillaceous sediments, treated by a graphical method of age calculation.

It has been noted that clays from the Gulf Coast of America⁴⁻⁶, pelagic sediments from the North Atlantic⁷ and from Pennsylvanian clays⁸, show a significant variation in the potassium content with grain size. There was an increase in the potassium content of the sample with a decrease in grain size. Size-fractionated samples of argillaceous sedimentary material selected from various stratigraphic horizons in the British Isles show this same trend (Table 1).

The variation in the potassium content with grain size could result from natural mineralogical variations which depend on grain size, for example, the coarse fractions could contain a higher proportion of non-potassium-bearing minerals such as quartz and calcite, 'diluting' the potassium content. However, even after the mineralogical variations have been taken into account, a systematic variation in the potassium content of the different grain sizes remains as seen in the clay corrected K₂O% (Table 1).

The variation in the potassium content with grain size may be due to potassium fixation which took place some time during the geological history of the sediment. The most probable time would be when most chemical changes took place during sedimentation, that is, at the time of diagenesis.

Table 1 Calculated mineralogical variations of argillaceous sediments from the British Isles

Sample reference no.	K ₂ O%	% Clay	Clay corrected	Age calculation by the standard method (Myr)	Isochron age (Myr)
KS2B	2.30	42.2	5.45	371 ± 6	315 ± 8
KS2C	3.36	48.6	6.91	386 ± 6	
KS2D	4.18	63.8	6.55	369 ± 6	
KS2E	4.63	77.0	6.01	359 ± 6	
KS2F	5.03	76.1	6.61	335 ± 5	296 ± 7
KS4A	1.01	75.5	1.34	349 ± 5	
KS4B	0.83	83.3	1.00	351 ± 5	
KS4C	2.28	81.3	2.80	353 ± 5	
KS4D	3.43	83.8	4.09	327 ± 5	355 ± 5
KS4E	4.33	87.6	4.73	294 ± 4	
KS12A	2.74	19.2	14.27	292 ± 5	
KS12B	3.71	51.6	7.19	277 ± 5	
KS12C	4.18	70.8	5.90	263 ± 5	236 ± 4
KS12D	4.47	76.2	5.87	256 ± 4	
KS12E	4.32	83.4	5.18	251 ± 4	
KS12F	3.84	87.7	4.38	236 ± 4	

Sample reference no. KS2, Coal Measures shale, grid reference SK 462 833; KS4, Permian Marl Slate, grid reference NZ 341 417; KS12, Triassic Marl, grid reference SK 601 060. Sample suffix A, 31–53 µm (4.25–5 Phi); B, 7.8–31 µm (5–7 Phi); C, 2.0–7.8 µm (7–9 Phi); D, 0.98–2.0 µm (9–10 Phi); E, 0.49–0.98 µm (10–11 Phi); F, <0.49 µm (11–13 Phi).

The finer particles contain more potassium than the coarser particles, as finer particles have a larger surface-to-volume ratio than the larger particles and they therefore acquire relatively more potassium during diagenesis.

The samples carry a record of the last major heating and cooling event, the orogenic date, at the centre of each grain (in the form of a core) and this core is surrounded by a mantle of material which carries the chemical and isotopic effects of diagenesis and thus the date of diagenesis. Transmission electron microscope views of clay particles show haloes of diagenetic alteration such as the Coal Measures shale KS2F in Fig. 1.

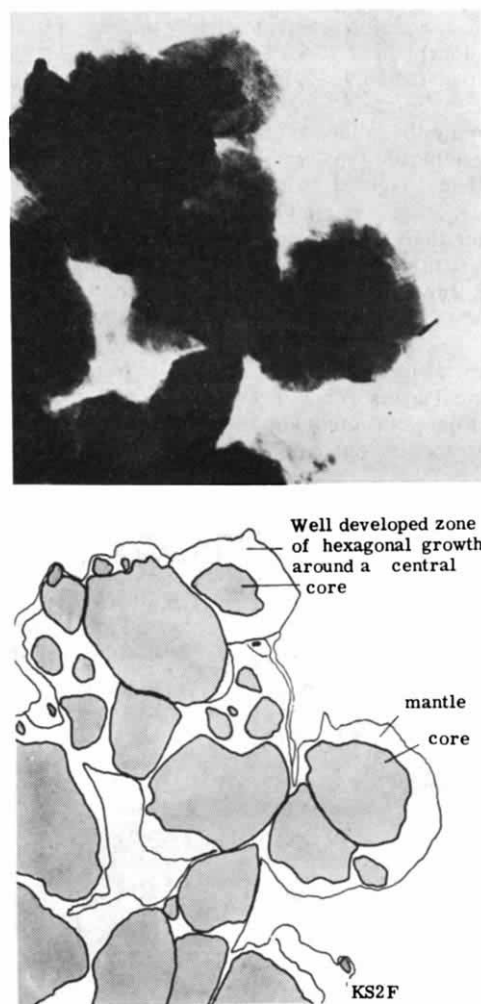


Fig. 1 Transmission electron microscope view of the 0.49 µm size fraction of Coal Measures shale, KS2F (× 18,900).

The graphical treatment of K-Ar data involves plotting an isochron with $^{40}\text{Ar}/^{36}\text{Ar}$ against $\text{K}_2\text{O}/^{36}\text{Ar}$ values. The gradient of the isochron is related to the age of the material assuming uniformity of non-radiogenic $^{40}\text{Ar}/^{36}\text{Ar}$ in each sample⁹. A standard isotope equation for an igneous sample is¹:

$$\frac{^{40}\text{Ar}}{^{36}\text{Ar}} = \frac{^{39}\text{K}}{^{36}\text{Ar}} \cdot \chi [\exp(\lambda_E(t_p - t_0)) - 1] + \frac{\text{Atm } ^{40}\text{Ar}}{^{36}\text{Ar}}$$

This is modified for sediments which have gained potassium during diagenesis;

$$\frac{^{40}\text{Ar}}{^{36}\text{Ar}} = \frac{^{39}\text{K}}{^{36}\text{Ar}} \cdot \chi \cdot (\exp[\lambda_E(t_p - t_0)] - 1) + \frac{^{39}\text{K}_0}{^{36}\text{Ar}} \cdot \chi \cdot (\exp[\lambda_E(t_p - t_0)] - \exp[\lambda_E(t_p - t_1)]) + \frac{\text{Atm } ^{40}\text{Ar}}{^{36}\text{Ar}}$$

where: ^{40}Ar , ^{40}Ar present in the sample; ^{36}Ar , ^{36}Ar present in

the sample; ^{39}K , average ^{39}K present in the sample; $^{39}\text{K}_0$, ^{39}K present in the core of the sample; $\text{Atm } ^{40}\text{Ar}$, atmospheric ^{40}Ar present in the sample; χ , the amount of potassium present as ^{40}K divided by the total amount of potassium expressed as the oxide = 9.897×10^{-5} ; λ_E , the decay constant for ^{40}K to ^{40}Ar = $0.585 \times 585 \times 10^{10} \text{ yr}^{-1}$; t_p , the present date; t_1 , the date of diagenesis; t_0 , the date of the orogeny. The last two terms in the equation are constant for a given sample. Thus, a plot of $^{40}\text{Ar}/^{36}\text{Ar}$ against $\text{K}_2\text{O}/^{36}\text{Ar}$ produces an isochron the gradient of which yields the time since diagenesis.

The isochron method of calculation does not require the assumption of the atmospheric ratio figure (295.5 or the instrumental equivalent¹⁰) and can be used to date samples which contain excess or inherited argon⁹.

With the exception of some work of glauconitic sediments, which were not sized¹¹, there has been no attempt to apply isochron interpretation techniques to K–Ar data from sediments. Workers who have sized sediments and obtained K–Ar dates by the standard calculation have assumed that the dates obtained from the very fine sediments tend towards the true date of diagenesis^{5,6,12}.

The results from the isochron analyses of size fractions of three sediments are shown in Fig. 2 for a Coal Measures shale, Permian Marl Slate and a Triassic marl from the British Isles. All data were corrected to take account of dilution effects from non-clay minerals. The corrected isochron age of $315 \pm 8 \text{ Myr}$ for the Coal Measures shale (KS2), falls within the 325–280 Myr Carboniferous range of ages summarised by Fitch, Foster and Miller².

The $296 \pm 7 \text{ Myr}$ date for the Permian Marl Slate (KS4) from County Durham could be considered a little too old for a diagenetic date for a mudrock near the base of the Permian. However, it compares well with the Permo/Carboniferous dates obtained from igneous rocks, so that the Marl Slate may represent an earlier Permian transgression than hitherto suspected.

These isochron ages are thought to be acceptable as stratigraphic dates. If no potassium fixation had taken place during diagenesis then a Caledonian age of $\sim 400 \text{ Myr}$ would have been obtained for both the Coal Measures shale and the Permian Marl Slate as the particles originated in the Scottish Caledonian region. The dates suggest that diagenetic alteration took place soon after burial, possibly because at this stage of the rock forming process, the sedimentary particles were most susceptible to changes in equilibrium resulting from their new chemical environment. The Triassic marl, KS12, a red bed sample, was included for comparison, and illustrates that it is not easy to obtain isotopic information from some sediments.

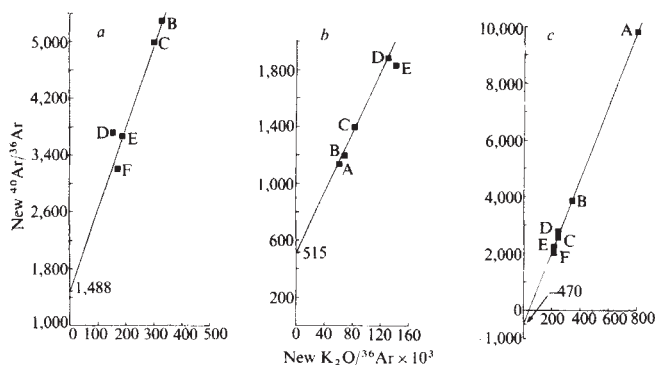


Fig. 2 K–Ar isochrons from a, Coal Measures shale KS2 (age 315 Myr); b, Permian Marl Slate KS4 (age 296 Myr); and c, Triassic marl KS12 (age 355 Myr). In each case the age is obtained from the gradient of the isochron line and the intercept on the ordinate relates to the excess or inherited argon in the samples. The fact that the intercept value is not 295.5 shows that the age dates obtained by the standard method of calculation would be erroneous.

The date $355 \pm 5 \text{ Myr}$ from the corrected isochron from this sample is clearly too old for the Triassic period, but is within the range obtained using K–Ar data on Cheshire Triassic sedimentary micas, also thought to have been derived from the Ammorican mountain chain³.

It seems possible that the arid palaeogeographical conditions prevalent in the Trias could have been responsible for a lack of potassium in the sedimentary environment and hence that a diagenetic chemical change involving potassium did not take place in the clays.

Isotopic information present in sediments could have wide applications in sedimentology and stratigraphy. In some circumstances the K–Ar method outlined here may be used to measure the age of sediments even though an inherited argon component is present.

The research was carried out at the University of Sheffield with the aid of a National Coal Board scholarship. I thank Drs J. G. Mitchell and C. D. Curtis, T. D. Ford and K. R. Pollitt for their help.

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Received 17 May; accepted 20 September 1978.

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Tectonic emplacement of ophiolitic rocks in the Precambrian Mona Complex of Anglesey

THE Mona Complex of Anglesey^{1–5} is a thick succession (over 5,000 m) of metasedimentary and locally metavolcanic rocks (the 'Bedded Succession'), gneisses of uncertain age and granite intrusions. The Monian Bedded Succession consists largely of flysch-type sediments deposited in a progressively shallowing sedimentary trough. The succession includes ferruginous chert and manganeseiferous shale, basaltic pillow lava and a serpentinite–gabbro suite of intrusions. The younger part of the succession has a mélange of regional extent, now interpreted as an olistostrome⁴. The sedimentary succession was complexly deformed and locally metamorphosed in high temperature/pressure (sillimanite–almandine facies) and low temperature/pressure (lawsonite–glaucofane facies) conditions, and intruded by granite during the latest Precambrian⁵. The structure and rock types present clearly indicate that the Mona Complex formed near to a late Precambrian destructive plate margin^{6–9}. In such a setting the association of deep ocean sediment, pillow basalt and a serpentinite–gabbro suite might represent progressively deeper oceanic crustal layers, and it has been proposed that these igneous rocks are fragments of the oceanic crust and upper mantle tectonically emplaced at the Monian destructive margin. However, recent detailed studies of the serpentinite–gabbro suite by Maltman^{10,11} suggest that these rocks were emplaced magmatically rather than tectonically (for example, ref. 11). Here, I review the character of the Monian ophiolitic association and argue that the serpentinite–gabbro

suite represents fragments of oceanic crust tectonically emplaced during oceanic subduction processes.

The serpentinites and gabbros occur within the sedimentary rocks of the New Harbour Group. This Group has a thickness of ~2,000 m and consists largely of rapid alternations of pelitic and pasammitic bands in varying proportions¹, suggestive of a moderate to deep depositional environment. The igneous rocks include serpentinites derived from dunite, harzburgite and lherzolite, ortho- and clinopyroxenite and websterite, and two-pyroxene gabbro^{1,8,10,11} occurring as small masses (up to 3 × 1 km), generally with surface outcrops of less than 1 km². They are well exposed in the Newborough district (G.R. SH 391635) but small outcrops also occur in North Anglesey, for example at Llanfechell (G.R. SH 364908) and Tre-gele (G.R. SH 357926). Contacts between the serpentinites and gabbros and sedimentary rocks are either obscured or are sheared. However, in the Newborough area, the igneous rocks are associated with a zone of epidote hornfels, which extends up to 300 m from the intrusive contact¹¹. For the well exposed Pwllpillo serpentinite-gabbro intrusion (G.R. SH 277770) it is clear from Maltman's map¹¹ that the epidote hornfels is only developed stratigraphically below the igneous rocks and is absent above.

The serpentinites and gabbros are completely restricted to the New Harbour Group, and never occur in older rock groups such as the South Stack Series. Further, within the New Harbour Group the serpentinite-gabbro suite consists of discontinuous outcrops around a single stratigraphic horizon; this is most clearly seen in the pattern of outcrop on South Holy Island around the Rhoscolyn anticline, but small outcrops at a similar level in the New Harbour Group in North Anglesey can be traced around the NWSE syncline centred on Llanrhywydrys. This pattern of outcrop is consistent with tectonic emplacement of serpentinite and gabbro fragments at a single horizon within the New Harbour Group, but is more difficult to explain as a result of magmatic emplacement. It seems likely that the present distribution of outcrops reflects disruption during emplacement together with effects of later folding.

The character and field relationships of the serpentinite-gabbro suite have led to contrasting hypotheses for emplacement of these rocks. Maltman^{10,11} has argued that the occurrence of finely preserved igneous textures, the absence of deformation of country rocks during emplacement, the occurrence of the zone of epidote hornfels around the serpentinite and gabbro, and the apparent absence of evidence for regional thrusting indicate that the serpentinite-gabbro suite cannot have been emplaced tectonically and that the ultramafic and mafic rocks were magmatic at the time of their emplacement. In contrast to this interpretation, the widespread occurrence of the features described by Maltman in many tectonically emplaced ophiolite complexes, allied with consideration of geological evolution of the Mona Complex, indicates that the serpentinite-gabbro suite is much more likely to be tectonically emplaced fragments of oceanic crust and hence part of an ophiolite complex in the modern sense (see ref. 12).

Many of the igneous features of the Monian serpentinite-gabbro suite characterise tectonically emplaced ophiolite complexes¹². Similarly, and as suggested in ref. 10, the apparent absence of syn-emplacement deformation also characterises many 'Tethyan' ophiolites which have little minor or major deformation associated with emplacement^{13,15}. The occurrence of metamorphic rocks associated with the serpentinite-gabbro suite is also believed to be characteristic of tectonically emplaced ophiolites. Woodcock and Robertson¹⁴ have reviewed 'ophiolite-related' metamorphic rocks of the Tethyan belt. These metamorphic rocks are commonly up to greenschist facies and occur up to several hundred metres from the ophiolite complex. They are "always in a similar structural position along or near to the contact of ophiolite with the underlying sedimentary-volcanic rock"¹⁴. The metamorphic rocks are generally "directly overlain by ultramafic or serpentinised ultramafic rocks, never by higher levels in the ophiolite stratigraphy"¹⁴. Woodcock and Smith¹⁴ attribute metamorphism to frictional heating

and residual heat from the parent oceanic crust. The petrological and structural features of the Mona ophiolites described by Maltman^{10,11} are therefore closely analogous to younger tectonically emplaced ophiolites such as those of the Tethyan belt.

Chemical analyses of serpentinite and gabbro samples indicate close similarity in major and trace-element composition particularly the high Mg/Fe, Cr and Ni, and low Ti, Na and K, between the samples and identical rock types dredged from oceanic ridges (see ref. 8). Similar studies of basaltic rocks from the younger Gwna Group (refs 7, 8 and unpublished data) clearly indicate an oceanic tholeiite character for the pillow basalts and the basaltic glaucophane schist. The oceanic character of these rocks indicates that they are likely to be allochthonous and, taken with their close association with an olistostrome, they provide evidence of local rapid uplift, erosion and transportation of fragments of oceanic crust^{4,9,16}. This evidence therefore indicates that tectonic emplacement of the serpentinite-gabbro suite is entirely feasible and is consistent with the geological history of the Mona Complex.

The evidence reviewed above is considered to indicate tectonic emplacement of the serpentinite-gabbro rocks into the New Harbour Group. The basaltic rocks within the New Harbour Group might also have been tectonically emplaced, or might have slumped into position like the components of the Gwna mélange. A problem in accepting these rocks as parts of an ophiolite complex in the modern sense¹² lies in the absence of a sheeted dyke complex^{10,11}. However, consideration of the processes of formation of oceanic crust indicates that with increase in spreading rate, the dyke swarm unit of the oceanic crust diminishes in thickness, and at very high spreading rates might be completely absent¹⁷. This possibility is consistent with the occurrence of ophiolite complexes in which the dyke swarm is lacking. For example, the Papuan ophiolite which seems to be rooted in oceanic crust apparently lacks a dyke swarm unit^{18,19}. In this respect, it is relevant that the chemical composition of some of the basaltic rocks in the Gwna Group (ref. 7 and unpublished data) can be used to infer a high spreading rate for some Monian oceanic rocks, using the correlation of TiO₂ concentration with spreading rate established by Nisbet and Pearce²⁰.

In conclusion, the geological features of the serpentinite-gabbro suite of the Mona Complex are consistent with formation as part of late Precambrian oceanic crust, followed by tectonic emplacement into the New Harbour Group. The occurrence of deep ocean sediment and oceanic pillow basalt (tectonically emplaced or slumped?) indicates that the Mona Complex has a disrupted and incomplete ophiolite complex which was emplaced into the New Harbour Group during the late Precambrian subduction in the area.

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Received 12 June; accepted 7 August 1978.

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Submerged early Holocene barrier reef south-east Florida shelf

AN extensive *Acropora palmata* (Lamarck) barrier reef flourished off the south-east coast of Florida in an area considerably north of present-day reef development during the early Holocene transgression. Pipeline construction across the continental shelf 40 km north of Miami (26°15'15"N, 80°03'52"W) has disclosed this relict shelf-edge reef that is at least 95 km long. Radiocarbon dates indicate that reef growth terminated ~7,000 yr ago, which coincides with the time of extensive flooding of the continental shelf. This relict barrier reef forms a low (10-m relief) ridge—the crest of which occurs at depths of 15–30 m—extending along the shelf break from Palm Beach southwards to Miami^{1,2}. Present-day fauna on the surface of the ridge include alcyonarians, sponges and scattered coral heads^{2,3}. The study reported here indicates that the early Holocene provided favourable conditions for reef development in the western Atlantic.

Five distinct facies⁴ were identified from samples obtained during excavation of a ditch (3 m wide, 450 m long, maximum depth 11 m) perpendicular to the trend of this shelf-edge feature: (1) back-reef coral head; (2) back-reef *A. cervicornis* (Lamarck); (3) *A. palmata*; (4) fore-reef coral head; and (5) fore-reef rubble. *A. palmata*, the dominant framework builder, is replaced by a large deposit of *A. cervicornis* in the immediate back-reef area (Fig. 1). The distribution of exposed reef facies suggests that reef zonation was similar to that of the modern Florida reef tract^{5,6}.

Radiocarbon dates (by Smithsonian Institution Radiocarbon Laboratory) from nine samples of unaltered skeletal aragonite range from 7,145 ± 80 yr BP near the top of the *A. palmata* facies, to 9,440 ± 85 yr BP at 10.5 m depth within the same facies. The position of dated *A. palmata* material compares favourably with information from sea-level curves now accepted for this part of the western Atlantic^{7,8}.

Four vertical sequences of dated samples (Table 1) indicate that the *A. palmata* facies accumulated at a mean rate of 6.6 m kyr⁻¹, with a maximum accumulation rate of 10.7 m kyr⁻¹. Recent studies have reported axial growth of *A. palmata* measuring from 40 mm yr⁻¹ off Panama⁹ to 8.3 mm per 30 d off St Croix¹⁰. These data, along with reported maximum accumulation rates of 10.8 m kyr⁻¹ (ref. 9) and 15.2 m kyr⁻¹ (ref. 11), suggest that *A. palmata* accumulation recorded in the Florida feature is not unexpected for an *A. palmata* reef which is capable of keeping pace with rising sea level^{7,8,12}. The internal structure of the early Holocene reef north of Miami reveals a growth history of rapid accumulation of *A. palmata* framework in response to a rapidly rising sea level, and the transition from a fringing reef to an extensive shelf-edge barrier reef following the submergence of the back-reef shelf margin (Fig. 1).

The death of the shallow-water *A. palmata* reef off Florida ~7,000 yr ago coincides with extensive flooding of large, relatively flat areas of the Florida continental shelf (Fig. 1) by rising seas of the Holocene transgression. Similarly, extensive shelf flooding has been correlated with the demise of a shelf-edge *A. palmata* reef off St Croix, thought to have been killed by turbidity derived from erosion of shelf soil cover¹³. Furthermore, turbidity related to erosion of shelf regolith is said to have prevented continuous Holocene reef growth in the eastern Caribbean on wide shelves at depths below 20 m (ref. 14). Many submerged ridges in the eastern Caribbean have been described as Holocene or Pleistocene relict reefs¹⁵.

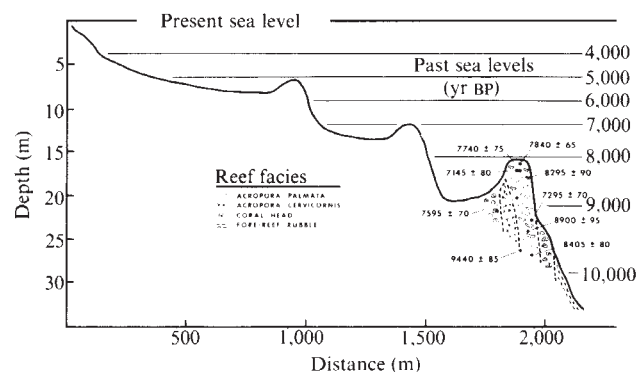


Fig. 1 Schematic profile¹ of south-east Florida shelf and cross section of relict barrier reef showing location of nine radiocarbon-dated samples and distribution of five reef facies. Plotting of Neumann's sea-level curve⁸ shows rapid reef-framework accumulation in response to rapid rise in sea level. Death of barrier reef ~7,000 yr ago coincides with extensive flooding of shelf.

Off southern Florida, the absence of a thick soil cover during the Pleistocene^{16,17} and the narrow shelf adjacent to the reef complex north of Miami limited the source of fine sediments during the flooding of this shelf. However, large quantities of fine sediment recently transported along the shelf edge north of Miami by the Florida current—following storm effects of hurricane Donna in the Florida Keys¹⁸—suggest that a major influx of turbid waters during the Holocene transgression could have originated from the south, where soil and lagoonal deposits of fine lime mud were widespread¹⁹. Such conditions ~7,000 yr ago would have exceeded the tolerance limits of the barrier reef *A. palmata* community.

Although the Florida and St Croix relict reefs are comparable in terms of composition, location at the shelf edge, and time of demise, present-day water temperatures in these areas differ drastically. Water temperatures off St Croix are never below the

Table 1 Age and vertical accumulation rates of *A. palmata* (Lamarck) reef samples

Vertical reef sequence	Depth in section (m)	Depth below present sea level (m)	Interval between samples (m)	¹⁴ C age (yr BP)	Age difference between samples	Accumulation rate (m kyr ⁻¹)
A	1.5	17.5	3.0	7,145 ± 80	450	6.7
A	4.5	20.5		7,595 ± 70		
B	0.5	16.5	10.0	7,840 ± 65	1,600	6.3
B	10.5	26.5		9,440 ± 85		
B	1.5	17.5		7,740 ± 65		
C	0.5	18.0	6.5	8,295 ± 90	605	10.7
C	7.0	24.5		8,900 ± 95		
D	0.5	23.0	4.0	7,295 ± 70	1,110	3.6
D	4.5	27.0		8,405 ± 80		

lower limits (generally accepted as 18°C) for active reef growth, whereas bottom temperatures along the eastern Florida shelf reach a minimum of 10°C during March, April and May²⁰. Warmer water temperatures off the eastern Florida shelf during early Holocene time are indicated by several exclusively tropical bryozoans (such as *Hippopodina feegeensis* (Busk)) and several tropical corals (including *A. palmata*) found within the reef facies of the relict reef. Therefore, cold water temperatures—which account for the absence of *A. palmata* reefs north of the modern Florida reef tract—may date back to the flooding of the shelf, and together with turbidity could have played a part in the death of the relict feature off Florida.

Vaughan similarly concluded in 1916 that reduced water temperatures prevented reef growth north of the modern Florida reef tract²¹. Vaughan did not identify a source for these cold waters, but he previously²² attributed many local geological processes to a southwards 'Florida countercurrent'. More recent studies^{23,24} also considered this countercurrent to be an influence on the modern Florida reefs, even though—aside from southerly littoral drift—it has been shown²⁵ that no such countercurrent exists. The continental shelf water in this area does interact with the meandering edge of the Florida current, frequently generating large southbound cyclonic spin-off eddies of warm water, warmer than resident shelf water, which affect large portions of the south-east Florida shelf (10–30 km) for several days^{25,26}.

Nevertheless, pronounced temperature fluctuations and unusually cold bottom waters in the shallow waters off south-east Florida are effectively preventing active reef growth north of Miami. This seasonal atmospheric cooling of shallow shelf-water which is significant today was probably more effective in reducing water temperatures—immediately following the extensive flooding of the Florida continental shelf ~7,000 yr ago. The large expanses of very shallow partially restricted incipient shelf-water (back-reef, inner shelf) of early Holocene time would have been easily cooled during the passage of winter storms and atmospheric cold fronts. Present-day unusually cold water temperatures off eastern Florida also occur when winds to the north produce an offshore Ekman transport of surface waters which results in the advection of cooler waters (upwelling), on the shelf for the duration of the wind event^{26–28}. Cold waters, whether advected on the shelf or atmospherically cooled, are transported only short distances southwards along the south-east Florida shelf incorporated with the trailing band of shelf water coupled to southward-bound spin-off eddies²⁹.

The highly variable currents and other transient properties produced by dynamic physical–oceanographic processes along this narrow continental shelf may have existed since the flooding of the shelf. Rapid flooding of the continental shelf during the Holocene transgression probably produced periods of extensive turbidity and rapid temperature fluctuations which led to the death of the shelf-edge barrier reef. Present-day deleterious physical–oceanographic processes along the south-east Florida shelf have apparently prevented re-establishment of *A. palmata* on otherwise suitable substrates north of Miami.

The shallow-water, early Holocene barrier reef off Florida has been effectively masked by a highly altered, submarine lithified, reef framework crust having a scattered, deeper water epifaunal cover. Exposure of this relict reef considerably north of present-day Florida reefs, sheds new light on the Quaternary reef history of south Florida. More important, this study supports findings off St Croix¹³ which, unlike the generally accepted hypothesis^{30–32}, indicate that early Holocene conditions were favourable for reef development in the western Atlantic.

Note that if relict shelf-edge reefs had survived flooding of shelf areas in the western Atlantic, barrier reefs adjacent to deep oceanic waters would be common rather than rare occurrences in this area.

Reef sampling was supported by a SEED grant to Florida Atlantic University geology department, diving assistance was provided by E. M. Clark, Jr, and diving facilities and cooperation by Powell Bros, Fort Lauderdale, Florida. This work was

supported partly by a Smithsonian Institution research fellowship. W. H. Adey, A. C. Neumann, and R. D. Perkins reviewed the manuscript, and A. H. Cheetham identified bryozoans in our samples.

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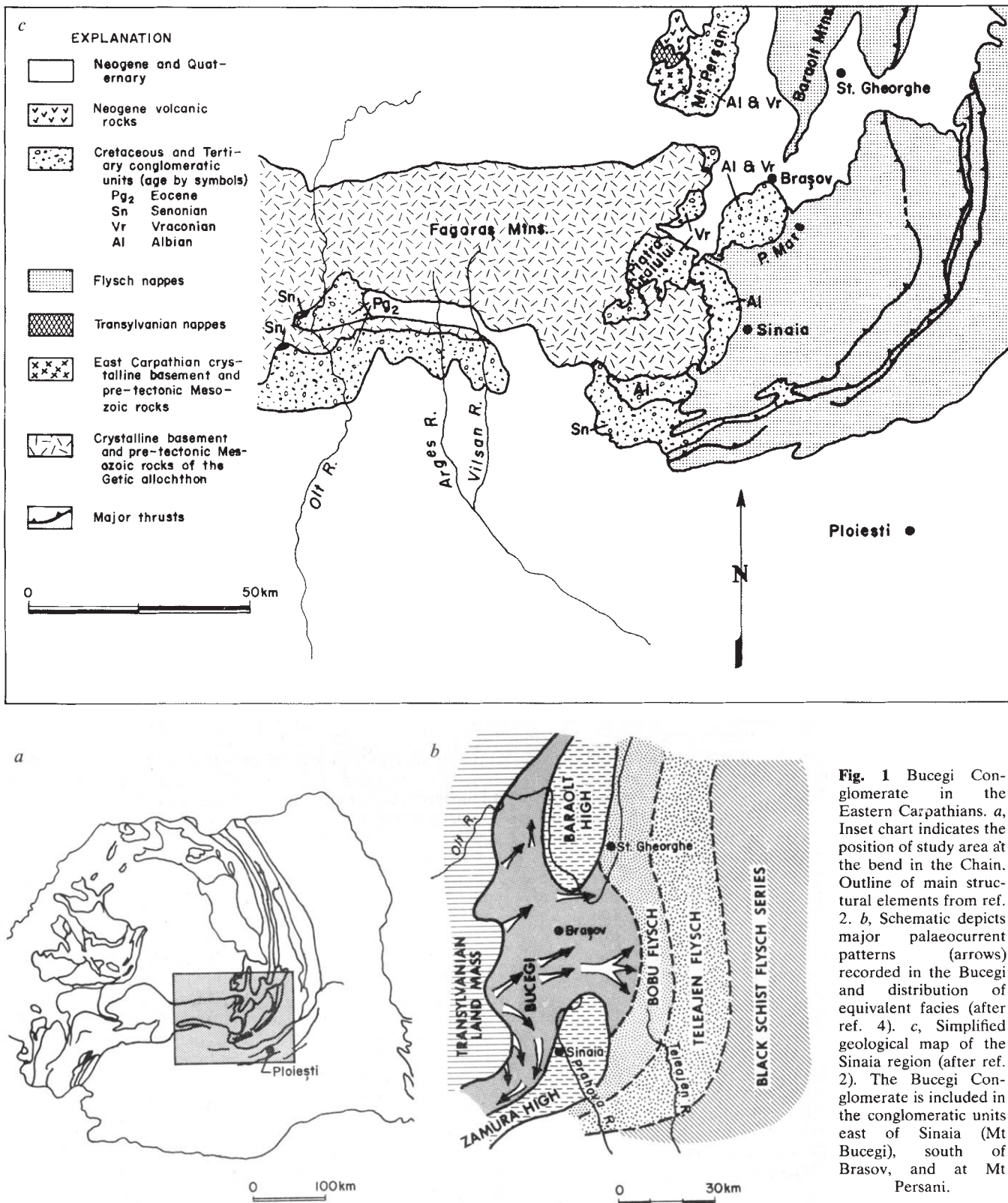
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Received 22 May; accepted 4 September 1978.

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The Bucegi Conglomerate: a Romanian Carpathian submarine slope deposit

THE Bucegi Conglomerate, a thick conglomerate-sandstone unit of Albian age (Cretaceous), is well exposed in the Eastern Carpathian Chain near Sinaia, Romania. This unit occupies more than 5,000 km² in the vicinity of the change in structural-stratigraphic trend of the mobile belt from E–W to N–S (Fig. 1a). The thickness of this remarkably coarse series is regionally variable and locally exceeds 2,000 m. To the west the conglomerate overlies, with angular unconformity, Middle and Upper Jurassic platform carbonate rocks which in turn unconformably overlie crystalline basement. To the east, over a distance of approximately 10 km, the Bucegi rests with local unconformity on Neocomian and Barremian–Aptian flysch several kilometres thick (Fig. 1b and c). This unconformity post-dates pre-upper Aptian to post-lower Aptian folding^{1,2}. Previous work has alluded to the shallow marine and nearshore



origin³ of this formation, and it has been frequently referred to as a molasse, or para-molasse⁴, mainly due to its coarse nature, its pebble shape^{3,5}, its stratigraphic position above sandstone-shale turbidite-rich formations, and presumably also to its 'post-tectonic' position within the mobile belt. Here we consider an alternative, that is that the Bucegi Conglomerate accumulated in an infra-neritic to deep marine environment and that it records progradation of coarse material directly onto the slope and base of slope of a turbidite basin. The field observations we made in conjunction with Romanian colleagues in selected sections of

the Sinaia region (Jepi Valley, Chindichiu road cuts) and an evaluation of previous reports provide a plausible basis to reconsider the depositional origin of the Bucegi.

The internal position of the Bucegi within the Eastern Carpathians, rather than the traditional, external fore-deep position in an orogenic belt, suggests that the conglomerate is not a molasse^{4,5}. A further problem with a molasse designation for the Bucegi Conglomerate is its temporal relationship to Carpathian tectonics. Emplacement of the crystalline rocks and Mesozoic platform rocks of the Getic allochthon (Fig. 1c), on

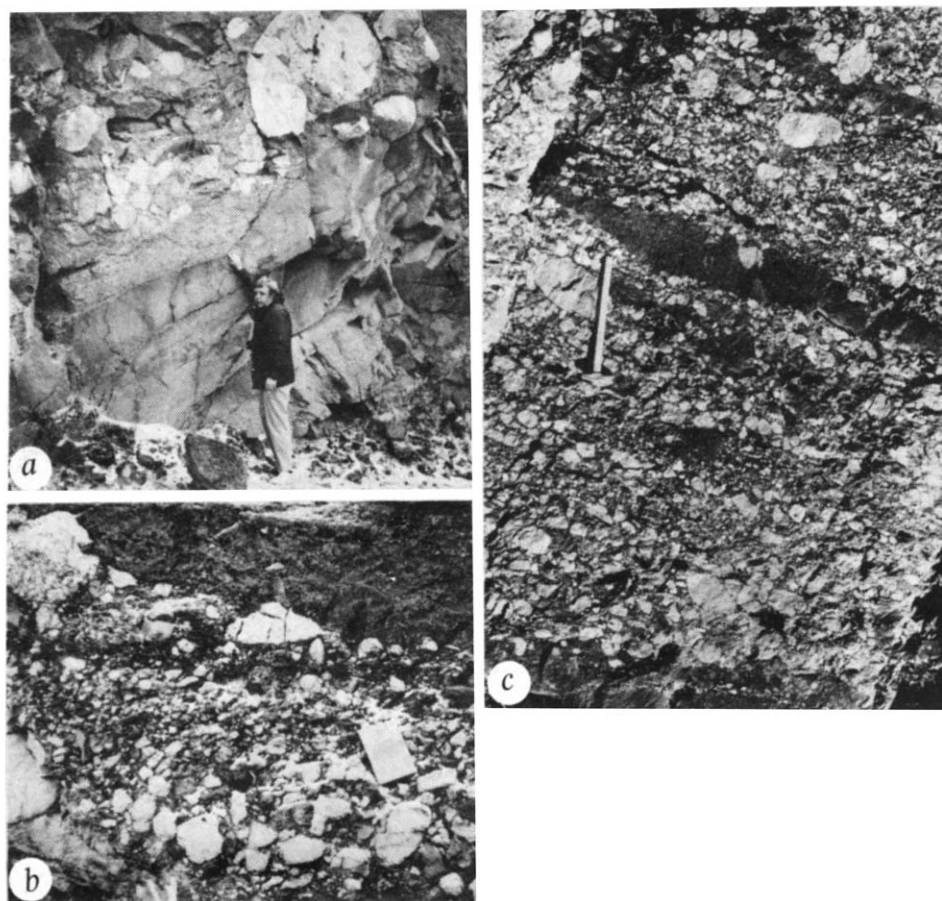


Fig. 2 Photographs showing selected Bučegi lithofacies in the Sinaia region. *a*, Thick, chaotic facies along the Chindichiu road cut above Sinaia. Note large clasts dispersed in a sandstone matrix and erosional base of conglomerate stratum cut into underlying sandstone bed. *b*, Graded diamictite displaying some pebble imbrication (book = 20 cm); Chindichiu road section. *c*, Well stratified sequence of moderately to well rounded limestone (mostly Cretaceous) pebbles; some strata display imbrication (hammer is 40 cm). Dark layers are muddy sandstone. Jepsi Valley section above Sinaia.

which the western margin of the Bučegi was deposited, must pre-date Bučegi (Albian) deposition. Except for this event (and the probably Barremian–Aptian emplacement of some of the Transylvanian nappes), eastwards thrusting of the crystalline and flysch nappes occurred later (post-Albian–pre-Vraconian and early and late Tertiary respectively)². Thus, the Albian Bučegi conglomerates predate intense Eastern Carpathian tectonic activity. We therefore avoid the term *molasse* which lends itself to considerable semantic ambiguity. Instead, an evaluation of the deposition of this formation is made in terms of (1) the tectono-stratigraphic and palaeogeographic evolution in this sector of the chain and (2) its sedimentological characteristics.

Interpretation of Bučegi Conglomerate deposition must account for the complicated relationships between it and: (1) the pre-tectonic crystalline rocks to the west and their pre-tectonic Mesozoic carbonate platform cover; and (2) the thick Neocomian and Barremian–Aptian flysch section to the east (Fig. 1*b* and *c*). Both these series are overlain unconformably by the Bučegi, yet to the south of Mt Bučegi the Barremian–Aptian rocks unconformably overlie the pre-tectonic crystalline rocks. At the eastern margin of Mt Bučegi the pre-Bučegi flysch section is ~2–3 km thick².

Burchfiel proposed a tectonic model to explain this close (<10 km) juxtaposition of the crystalline-carbonate platform rocks with the thick flysch sequence. A slightly modified scheme follows. In late Jurassic time a structural-morphological high existed on the carbonate platform to the west; from this, a slope extended eastwards into the evolving Eastern Carpathian flysch basin. Eastwards thrusting of the crystalline and overlying sedimentary series (Getic allochthon) onto the slope in Neocomian time resulted in placing olistoliths of crystalline and other lithologies into the Neocomian Sinaia Beds of the flysch basin. Thrusting (and possibly large-scale sliding) in an eastwards direction continued in Barremian–Aptian time so that

turbidite-rich units (flysch) of this age were in part overridden and folded by the crystalline thrust and were locally deposited on the thrust plate. Olistoliths of Mesozoic limestone were shed into the Barremian–Aptian flysch. During the Albian, Mesozoic platform carbonates were eroded, transported eastwards and redeposited across the thrust front as the Bučegi Conglomerate. As shown by Contescu⁶ these conglomerates stratigraphically may represent the proximal facies equivalents of the Bobu flysch and more distal Teleajen flysch. The Bobu flysch consists of 2,000–3,000 m of platy sandstone, marly shale, and massive sandstone in the lower part. The upper part includes a massive sand and conglomerate (Bučegi type) complex capped by micaceous, marly sandstone¹. The Teleajen flysch consists of 'curbicortical' (convolute laminated) sands alternating with marly and sandy argillites. In the upper part are intervals of thick or massive sands, in part conglomeratic¹.

Evidence suggests that the Bučegi was deposited on, and at the base of, a submarine slope in water of infraneritic to bathyal depth rather than in a shallow marine and near coastal environment as earlier suggested^{4–6}. These include: (1) the Bučegi apparently is the proximal facies equivalent of the Bobu flysch (ref. 4, Fig. 5; ref. 6, Fig. 13). The Bobu flysch contains thick bedded, coarse turbidite sandstone and conglomerate of the Bučegi type^{1,7}. (2) The area covered by the Bučegi conglomerates is ~100 km long and 50 km wide and palaeocurrent directions radiate in fan-like fashion⁵ (Fig. 1*b*); the coarseness and palaeocurrent pattern suggest limited dispersal and proximity to source terrains. (3) Large olistoliths derived from the west are present in both the Neocomian and Barremian–Aptian units below the Bučegi, are present in Aptian–Albian sandstone and conglomerate in the eastern Piatra Mare north of Mt Bučegi², and are present in Vraconian–Cenomanian conglomerate overlying the Bučegi at Piatra Craiului immediately west of Mt Bučegi². (4) Albian rocks interpreted as possible slope deposits (Patrușiu, in ref. 2) occur immediately north

of Mt Bucegi. (5) Slump structures and olistoliths (up to 80 m long) are present within the Bucegi conglomerate indicating the presence of a marked gradient east of the emerged terrain⁷. Thus, both stratigraphic and palaeogeographic considerations strongly favour a slope origin for the sedimentary series deposited below, above, adjacent to, and in the Bucegi. We know of no evidence to indicate that the depositional environment of the Bucegi was anomalous (of shallow coastal origin) in this respect.

A deep-water origin of the Bucegi is also suggested by the assemblage of sedimentary structures and fabric displayed by the sandy conglomerates. The series is characterised primarily by a marked bed-to-bed variation in terms of (1) thickness (some strata exceed 10 m); (2) the relative proportion of boulders, pebbles, sand, and to a lesser extent mud; and (3) the composition. Strata include chaotic mixes (diamictites) of unsorted gravel, sand, and mud (Fig. 2a); poor to well developed graded (Fig. 2b) and reverse-graded units; and moderately to well stratified sandstone conglomerates, some of which show pebble imbrication (Fig. 2c). In the latter, clast shapes and the internal stratification of some imbricated pebble beds together could suggest a fluvial or a coastal origin⁵. Boulders locally exceed 150 cm in diameter (Fig. 2a). The coarse clasts include a highly varied polymict assemblage of Mesozoic and older sedimentary and metamorphic rocks⁵; a large proportion of these are subspherical to elongate and moderately to well rounded. Characteristically, the clasts are dispersed ('float') in a sandstone-granule or muddy sandy matrix; pebbles, cobbles, and boulders are uncommonly in contact although the matrix-to-clast ratio varies greatly from bed to bed. Imbrication is best developed in some of the better stratified units (Fig. 2c). Shale clasts are locally present within the pebble units and these vary in size; their position within pebble beds is inconsistent. Linear, load-deformed sole markings including large (>50 cm) flutes (Fig. 3) are preserved at the base of some beds whereas other conglomerate units are sharp-based and truncate underlying units (Fig. 2a) without sole markings. Individual beds are areally-restricted blanket to wedge-shaped units that occasionally display a channel geometry. Few of the sandy conglomerates may be defined as sheets.

Fig. 3 Large flute marks (>50 cm) on the base of the first thick conglomerate layer in the basal Bucegi (Jepi Valley above Sinaia). Palaeocurrent direction from lower right to upper left. This unit eroded the underlying turbidites of Barremian–Aptian age.



Various mass-flow processes may account for the majority of thicker coarse, chaotic units with poorly defined fabric. The most likely sediment gravity flow mechanism capable of displacing boulders is debris flow which involves clast-support mainly by fluid-matrix strength as determined in experimental studies⁸. Debris flows may involve slow displacement of mud- and sand-supported coarse clasts as observed in upper canyon reaches^{9,10}, although large flutes at the base of some Bucegi beds indicate that some flows were rapid. It has been suggested that graded conglomerates can be transported by turbulent flows^{11–14} where large clasts are maintained in suspension by fluid turbulence. Dispersive-pressure flows (grain flows) may produce inverse grading. Traction-produced features in stratified conglomerate layers also may indicate layer-by-layer deposition from turbulent flows as discussed by Winn and Dott¹³. The fabric and chaotic orientation of some large clasts indicate that down-slope movement by sliding, rolling, and creep were probably also involved.

Deep-water pebble and boulder transport mechanisms admittedly remain poorly understood. The Bucegi features observed probably represent the interplay of several grain support mechanisms during flow as theoretically depicted by Middleton and Hampton¹⁵. They emphasised that a gravity flow may, during various stages of downslope movement, combine several mechanisms. We believe that this phenomenon of downslope transformation would best explain the regional facies distribution in the Eastern Carpathian Albian basin, that is coarse Bucegi conglomerates emplaced primarily on the slope between the upper margin and base-of-slope, including proximal fan environments and decreasing proportions of conglomerate and massive sandstone and enhanced proportions of sandstone and finer-grained turbidites in more distal (outer fan, basin plain) sectors. The scheme we envision thus includes by-passing of somewhat finer grained fractions seawards and a progressive textural fractionation beyond the base of the slope.

The deposition of large rounded clasts on a slope recalls present conditions on the Ligurian margin of the Mediterranean off the Maritime Alps where relatively short, but powerful, rivers cut across terrains of marked relief and deliver coarse deposits to the coast and to small deltas (the Var delta at Nice, for example). Both fluvial transport and reworking along the coast result in rounded and flattened pebbles. These clasts, with their inherited fluvial shape, prograde on mud and sand of the slope and extend seawards to the deep Ligurian basin plain; in contrast, the basin plain fill consists largely of mud and sand turbidites¹⁶. In favour of a similar slope-deep water model for the Bucegi are large olistoliths and slumped units locally incorporated in the boulder beds⁷. These record large-scale failure of series that formed slopes on the basin margin. The gravity sliding on the Albian basin margin of olistoliths, slumps, and mixes of sand and gravel may have been triggered by a combination of earthquakes and/or rapid accumulation of sand and gravel on the upper margin. Conditions such as these may cause the shearing resistance to be suddenly exceeded, with resultant collapse of the metastable structure and the initiation of flow on relatively steep gradients. Rivers, some of them large and torrential, drained emerging high-relief metamorphic and carbonate terrains to the west and carried sizeable volumes of mud to boulder size material to the coast. Pebbles, rounded during fluvial transport, were further modified during movement along the coast; sandy gravel deltas probably extended across the narrow shelf directly on to the upper slope. Pebbles and boulders were subsequently redeposited with sand by various mass processes onto the east-dipping submarine slope where they formed thick, coalescing deposits over broad sectors of the margin. The gravels are widespread and do not seem to have been restricted to submarine valleys; strata commonly record channelling, suggesting that slope and base-of-slope channel systems may have been braided¹³. The regional facies distribution patterns cited earlier suggest that significant proportions of mud and some of the sand introduced with gravels were differentially moved on the slope by mass processes,

including grain flows and turbidity currents, and entrained to more distal sectors of the basin than the pebble-rich sediment.

Other examples of pebble-rich deposits prograding above finer-grained, deep-water units on basin margins are recognised in the Carpathians (see Kamenica zone of Czechoslovakia discussed by Marchalko¹⁷). Most of the world's mobile belts, including the Alps¹⁸, Apennines¹⁹, Ouachitas²⁰, Andes^{13,21}, Appalachians¹², California^{22,23}, and New Zealand¹⁴, also display resedimented mass-emplaced conglomeratic series that were deposited on slopes and in some cases displaced to lower gradient base-of-slope, including fan and basin plain, environments. Possible modern analogues of deep water Bucegi type pebble-rich deposits emplaced by mass-flow processes are noted in canyons^{10,24}, on broad sectors of the slope²⁵, and in basin plains distant from source and coastal sectors²⁶.

Our interpretation of the Bucegi requires the rapid evolution of the structural-physiographic configuration and relief of the emerging hinterland west of the Albanian basin. Thus, the rapid transition from marine turbidite-rich to gravel series does not necessitate a marked change from deep to shallow water basin configuration as implied by the 2,000 m of conglomerate in the molasse model, but may reflect the importance of a newly developed fluvially-drained source adjoining the submarine margin. An examination of the pebble and boulder beds similar to the Bucegi that crop out west of the study area such as the younger Cretaceous and Palaeogene series of the Olt, Olanesti, and Vilsan River valleys (Fig. 1c) would be useful in refining the palaeogeographic reconstruction of this sector of the Carpathians.

We thank the US and Romanian National Academies of Sciences for arranging the exchange visits (1973, 1976) in Romania and D. Jipa, N. Mihailescu, N. Panin, and M. Sandulescu of the Romanian Geological Institute for valuable discussions. B.H. thanks the University of Maine summer faculty research fund for support in 1973. The paper was reviewed by B. C. Burchfiel, L. R. Contescu, M. A. Hampton and H. G. Reading.

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Can electromagnetism account for extra-sensory phenomena?

OUR investigations of various alleged extra-sensory phenomena (ESP) over the past three years are reported here, in particular psychokinesis, metal-bending, psychic healing and dowsing. One of our aims was to search for unusual electromagnetic (EM) radiation emitted by subjects while they were achieving or trying to achieve the phenomena. The quest for EM concomitants of ESP is based on our deduction that it is the only known force that could conceivably be involved (ref. 1 and J.G.T. and E. Balanovski, unpublished). In looking for EM signals emitted by people during alleged ESP events we are therefore testing the reality of the corresponding phenomena. There might be no paranormal phenomena at all, so that a search for abnormal EM effects would automatically fail. If we assume that the present evidence for ESP is not firm either way, then its EM characteristics are highly relevant to resolving that problem. If no EM signal were found, this would question the reality of the phenomena whereas suitably strong EM signals would support the claim that ESP effects were occurring. This can be quantified if the sensitivity of subjects to incoming EM radiation is ascertained; for example, sensitivity at least down to the levels of any paranormal emission from others would be necessary for telepathy to occur. We find no abnormal EM signals during the occurrence of supposed ESP phenomena.

Earlier work on EM signals related to ESP has been inconclusive. Cazzamali² proposed an EM explanation of telepathy by means of waves of 10 cm-1 m in wavelength, and he claimed to have detected such emission. These results were never confirmed. Vasiliev³ concluded that telepathy could not be explained by electromagnetism. A critical review of his work shows lack of statistical significance in his results, thus leaving the EM explanation still open. Various other tests⁴ have been put forward to contradict the EM explanation of ESP, but they all exclude some portion of the EM spectrum. It seemed appropriate for us to use suitably sensitive detectors to clarify the position of the EM hypothesis in ESP phenomena.

We used EM detectors covering a wide range of frequencies (Table 1) together with two video-tape cameras, one to keep a continuous record of the subjects' performance and the other to keep a permanent record of the visual readout of some of the detectors. The EM detectors consisted of various antennae with amplifiers producing either a visual display or a written record. For frequencies below 1 MHz both skin electrodes, usually taped to the palm or the wrist of the subject's hands, and wire antennae within 1 m of the subjects, were used. For frequencies in the range of 1 MHz-20 GHz (detectors P_2) two loop antennae were used⁷, one for frequencies below 500 MHz and the other for the upper range. Each antenna was placed against the palm of the subject's hand. For the microwave radiometers T_2 and T_3 horn antennae were used directly on the subject's body. The two crystal detectors P_4 (60-90 GHz and 90-140 GHz) had narrow slits which allowed emission for the subject's fingertips or palms to be measured by direct contact. Background signals above the noise levels of the detectors were picked up in various situations. In d.c.-1 MHz range (detectors E_1 , E_2 , E_3 and P_3) various external signals correlated with transient electric field disturbances produced by passing vehicles were noted at least 10 times above the noise level. In the 1 MHz-120 GHz range (on detectors P_1 , P_2 , P_3 , P_4 , T_1 , T_2 , T_3 , and T_4) no signals above noise levels (of 5 dB) were noted other than various narrow and assignable TV and radio stations (with clear and expected shape under high resolution Fourier analysis) and the expected human

black body emissions. This was detected (on detectors T_1 , T_2 , T_3 and T_4) with a noise level of 0.5 K. A similar level of sensitivity occurred for T_5 in the infrared range. H_1 was sensitive to 1 nT, and T_6 responded to a 10 W UV source.

The outputs were recorded either on strip chart recorders, tape recorders or direct photographs of the detector recording screen (oscilloscope, frequency analyser). The resulting records were then examined visually. Only if an abnormal signal had occurred which was above the noise level and not explicable in terms of external interference or natural human body EM emission, would more detailed signal analysis have been justified.

Because several detectors were spectrum analysers (see Table 1) we have obtained an upper limit to the power level radiated at various frequencies by a person allegedly performing ESP. We have only investigated integrated power levels, as enough power has to be radiated before a signal can achieve its desired effect, whatever modulation the signal may have. In all tests reported the subjects always claimed that they were in a good 'psychic' state. Thus our measurements can be taken as demonstrating the nature of human EM emission during so-called psychic activity. Despite that, we were unable to detect any abnormal EM emission from any of the subjects involved in the following tests.

Psychokinesis is the alleged phenomenon whereby a person is able to achieve movements of objects without physical contact.

(1) Needle-rotation (two subjects, two controls, 92 trials): a needle was suspended by an extra-fine nylon thread inside a clear perspex cylinder which was clamped securely to a rigid bench. Movement was achieved by the subjects moving their hands and wrists back and forth around the cylinder at about 5 cm from it. In each of the 12 sessions the subjects produced a 60° average rotation of the needle, sometimes up to 200°. The rotation built up gradually over the first half-hour of each session, and consisted of the needle rotating with the position of the subject's hands near the cylinder. The needle swung back to its equilibrium positions as the subject's hands moved back from the cylinder. Rotation was achieved both by the subjects and the control subjects.

We established that a rotation of 50° was produced by a potential of 2 mV with respect to earth at frequencies d.c.–1 MHz. For up to 60° rotation, measurements on two subjects, by means of skin electrodes (the subjects being earthed) indicated a skin potential of only 0.2 mV. We concluded that the rotation could not be caused by E or H fields from d.c.–1 MHz produced by the subject's body. When an anti-static ointment was rubbed over the surface of the cylinder, the whole phenomenon vanished, indicating that the effect was due to charge induced on the needle from the outer surface of the

cylinder. Analysis of the VTR of the events showed the subjects inadvertently touching the surface of the cylinder in their attempts to move the needle. Further tests showed that the amount of rotation produced was directly correlated to the amount of charge on the outer surface of the cylinder.

(2) Rotation of a straw inside a glass dome (one subject, eight trials): a piece of drinking straw cut in a T shape was fixed onto a light plastic disk and placed on top of a glass of water, full to the brim, enclosed inside a large glass dome (70 cm across). Rotation occurred, up to 20° over 7 s, with the subject sitting quietly, not bringing her hands near the dome. No abnormal levels of EM radiation from the subject were detected.

After half an hour, a mist developed on the wall of the dome further away from the subject, due to condensation of the water from the glass. This was traced to the heat from an electric fire located behind the subject. With the electric fire turned off no rotation occurred. Calculation of the convection current inside the dome with the fire on showed that it was more than sufficient to have caused rotation.

(3) Compass-needle rotation (two subjects, 36 trials): rotation of a compass-needle occurred when the subjects moved their hands back and forth, at a distance of 5–10 cm from the compass case.

EM detection from the subject's body showed no abnormal signals. The amount of rotation was found to decrease with increasing distance between the compass and the subject's body according to the inverse square law. It also decreased when steel sheets were placed between the subject and the compass needle. This indicates an electrostatic origin for the rotation.

Metal-bending (68 subjects, 268 trials): claims of metal-bending can be divided into two groups:

(1) contact bending, that is by stroking; and (2) bending at a distance.

Our experiments were carried out under four separate sets of conditions; in all cases remote EM detectors were present.

(1) A subject was asked to stroke a piece of metal of a length of up to 15 cm. The strip was securely attached to the top of a GPO balance and could only be touched on its upper surface. A video-tape camera recorded the strip, the finger stroking it and the pressure applied at all times. A clock showing the second hand was also included in the picture to assure continuity. (2) Electromyograph (EMG) sensors were placed on the subject's forearms, and a continuous picture of the dials of both EMG boxes was obtained with the VTR, thus giving a permanent record of the pressure applied by the subject. (3) A room with a one-way viewing system^{5,6} was used (these tests were done in collaboration with H. Collins, Bath University); the subjects were informed of the one-way system beforehand, the video-

Table 1 The sensitivity and time constants of the various detectors used in the tests as a function of frequency

Detector	Description of antenna and display used	Frequency range	Sensitivity	Resolution time constant
E_1	Skin electrodes or wire antennae connected to CRT display	d.c.–1 MHz	10^{-4} V cm ⁻¹	1 μ s
E_2	Skin electrodes or wire antennae connected to CRT display	d.c.–1 MHz	10^{-2} V cm ⁻¹	1 μ s
E_3	Electrometer connected to 10 cm ² metal plate—20 cm from subject	d.c.–1 MHz	10^{-4} V cm ⁻¹	1 μ s
H_1	Magnetometer and probe	d.c.–1 kHz	10^{-5} G	1 ms
P_1	Loop antenna and crystal detector—50 cm from subject	d.c.–1,250 MHz	1 V cm ⁻¹	1 ns
P_2	Loop antenna connected to broad band frequency analyser	1 MHz–20 GHz	10^{-3} V cm ⁻¹	1 ns
P_3	Skin electrodes connected to frequency analyser	d.c.–1,250 MHz	10^{-3} V cm ⁻¹	1 μ s
P_4	Crystal detectors (in contact with subject's hands) connected to microwave detector	60–140 GHz	1 V cm ⁻¹	1 μ s
T_1	Loop antenna connected to microwave radiometer	1.4 GHz	1 K	2 s
T_2	Horn antenna (contact with body) connected to microwave radiometer	15 GHz	0.5 K	0.5 s
T_3	Horn antenna (contact with body) connected to microwave radiometer	19 GHz	0.1 K	0.33 s
T_4	Thermocouple and electronic thermometer	3×10^3 – 3×10^5 GHz	0.1 K	1 s
T_5	Ge probe and chopper 1 m from subject (IR detector)	3×10^3 – 3×10^5 GHz	0.5 K	1 s
T_6	UV detector	0.2–0.35 μ Hz		0.1 s

The sensitivities were obtained directly by calibration using known external sources, and were evaluated for signals about 2 s.d. above the noise level. The time constants were as specified by the manufacturers. No detector was used at a distance greater than 1 m from the subject.

tape camera was placed on one side of the one-way mirror, while the subject sat on the other side and attempted bending. The GPO balance or the EMGs were used together with this set-up. (4) A more relaxed environment in which neither EMGs nor VTR were used was the only condition in which success was claimed.

Background levels were measured on all the detectors (E_1 , E_3 , H_1 , P_1 , P_4 , T_1) before and after tests and were as expected in the various environments. No signals above background were observed at any time in the whole frequency range d.c.– γ rays. In the lower frequency end of the spectrum about 180 trials were made with each detector, only 15 or so trials were effected at the higher frequencies.

No metal bending at all was obtained under the stricter protocols (1), (2), or (3) but only under the more relaxed environment of (4). This might be related to the psychological stress presented by the more restrictive regimes, as some of the subjects remarked. We gave them the benefit of the doubt and tested them also in their more accustomed setting, where they sometimes were apparently successful. We still obtained no EM signal, although at certain times in the more relaxed conditions (4) metal bending occurred (of paperclips, spoons, prepared strips and so on). Each of these successes took several seconds to minutes to occur.

Attempts were made to cause bending of a strip of metal or plastic, by feeding EM energy into the strip. A Paradyamics 10 GHz X-band microwave source was used (50 kW peak power, 0.6 or 2.1 μ s pulse, variable p.r.f. with external modulation). Strips of various metals, plastic and various crystals were irradiated, and vibration of the specimens was observed at the modulation frequency in agreement with surface acoustic wave generation^{8–10}. Strips of Al (length 63.4 cm), Cu (length 39.8 cm) and brass (length 51.6 cm) cut to these lengths appropriate to the internal modulation, showed resonance effects (at 1–3 KHz) when inserted in the waveguide of the X-band source. Although energy was thus absorbed in the strips, no bending ever occurred.

The results show that no unusual EM emission from the subject's body was observed over the entire spectrum. If there had been low frequency signals they could not account for the phenomenon as first, their focusing power is very poor, second, the energy transfer is inefficient, and third, the signal levels observed are too low by a factor of about 10^9 to explain the effect. The best candidate would be the microwave range 1–5 GHz; at these frequencies the focusing power is good and the energy transfer can be efficient for the generation of surface acoustic waves as the skin depth in metals at these frequencies is negligible. But no microwave emission higher than the black-body radiation at the human body temperature was ever detected. Microwave radiation emitted by the body corresponds to a power level of 10^{-14} W. Therefore, EM radiation cannot explain the above-mentioned metal-bending cases which only occurred in the relaxed conditions (4).

Psychic healing (six subjects, >12 trials for each detector): by 'psychic healing' we only mean 'laying on of hands' and not the more dubious psychic 'surgery'. Healers claim cures of terminal cancer and other illnesses apparently inaccessible to standard medical practice. Sensations of heat and cold are said to occur during laying-on of hands. Such sensations have been claimed both by the healer and by the patient.

No unusual EM radiation was observed in the range d.c.–140 GHz during 'healing'. The protocol was to attempt detection of enhanced EM levels from the subjects both when the patient was present and when being allegedly healed at a distance. Two video-tape cameras were also used. Detector T_4 was used as a broad-band infrared detector both for emission and sensitivity of the body to this radiation. This allowed us to investigate whether the sensations of cold or heat experienced by the healer or the patient could be correlated to actual decrease or increase of surface skin temperature in the subjects.

The procedure was to measure the temperature of the healer's hands as a function of time and to note what sensation (heat or

cold) the patient was undergoing, and what the healer was attempting (heat or cold). At no time were the temperature readings indicated to the healer or to the patient.

The sequences recorded (typically over 10–20 min) show that the healers had no clear control over the temperature of their hands. Nor were the patient's sensations in any way correlated with surface skin temperature. These sensations cannot be accounted for by subcutaneous heating, as this would involve radiation transfer in the MW or RF ranges; neither was ever observed.

Tests on human sensitivity to low levels of EM radiation (five subjects, two controls): a subject was seated in a room with either a loop antenna (lower frequency range) or a horn antenna (higher range) placed 50 cm from him. The source was in an adjoining room to avoid visual or auditory cueing, conscious or unconscious. The sources used were a tuneable RF source in the range 220–950 MHz with a power output of 1 mW and a tuneable MW source (both pulsed and CW) in the range 6–17 GHz with a power level of 5 mW. The switching on or off of the source was randomised by tossing a coin. The level of success according to chance was therefore 0.5. The subject was then asked to attempt to sense the source being on or off. The results showed that both control and test subjects achieved results not significantly different from chance. This insensitivity to low level EM radiation did not warrant further trials.

Dowsing: we investigated two dowsers for the possibility of EM emission where the 'dowsing reaction' occurred and for their sensitivity to EM radiation (active and passive radar-like type of mechanism). This 'dowsing reaction' is a sudden 'flicking' (up or down) of the rod or rotation of the pendulum when the dowser reaches a certain place at which the object being dowsed for is supposedly located.

During a positive dowsing reaction the EM radiation emitted by either subject from 1 MHz to 22.4 GHz was no different from background levels measured in the detectors before starting or finishing each session.

Telepathy—distant-viewing: telepathy can be defined as the ability of a subject to perceive another subject's thoughts. It involves the transmission of information between a sender and a receiver in a non-verbal fashion. We only investigated integrated power levels, as enough power has to be radiated before a signal can achieve its desired effect, whatever modulation the signal may have. We investigated three subjects who claimed telepathic abilities and one who claimed distant-viewing abilities, in which a subject is supposed to describe accurately a remote site without his being physically there. No EM signals other than background noise were observed on E_1 , E_3 and H_3 . Nor did the tests show evidence of the subjects being successful in telepathic transmissions.

We have tried to detect EM signals emitted by people, and in particular the Fourier spectrum of such signals, to test the reality of ESP phenomena. In particular, we have investigated many different subjects attempting to achieve ESP phenomena, but have failed to detect any unusual EM radiation. The absence of effects on UV detectors or photographic films completed the study of the higher range of the electromagnetic spectrum. We also investigated human sensitivity to low levels of EM radiation over a restricted range of frequencies and found no indication of humans being sensitive to those EM fields. Dowisers, who claimed sensitivity to magnetic fields, down to 10^{-5} G, were also tested and found insensitive to the presence of 100 G.

It is possible to conceive transmission of EM energy from one person to another or of emission by one person in a manner undetectable by the apparatus we have used. This would have been so if very brief pulses of EM energy were used in such signalling with times less than the response time of the corresponding apparatus at the frequency used. There are no known mechanisms in the body able to produce such signals at the power levels required to produce the effects. We have also found that humans are insensitive to low levels of EM. A possible mechanism for such signalling is therefore clearly ruled out for telepathy, distant-viewing and psychic healing. The EM

levels emitted to achieve metal bending (in the microwave range to achieve the desired focusing) are joules¹, and there is no known mechanism in the body to achieve a peak power output of GW; it is difficult to suppose that this would be possible without severe tissue damage.

We can distinguish four different categories of effects from our investigations: (1) the effect occurred but it could be explained in normal scientific terms (psychokinesis); (2) the effect occurred and could have been paranormal (dowsing, faith healing); (3) the effect occurred in less than perfect conditions (some metal bending trials); (4) the effect did not occur (other metal bending trials, telepathy, distant-viewing). The consistent absence of any unusual EM radiations associated with phenomena in all the above categories, in which all the subjects always claimed to be in a good psychic state, causes us to question the paranormal nature of those in categories (2) and (3), because we contend¹ that EM is the only known force that could have been involved in the phenomenon. We therefore conclude that phenomena of category (2) have their natural explanation, dowsing as muscular twitches brought about by subconscious mental activity, faith healing as the purely (albeit complex) psychological effect of the healer on the patient; in particular, metal bending does not come into this category (category (2)) in the cases we have investigated.

We thank the Sedwood Trust for financial support, all our subjects for their patience, and many colleagues for the loan of the equipment.

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Received 11 May 1977; accepted 20 June 1978.

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Newly discovered fossil hominid skull from the Afar depression, Ethiopia

DURING a palaeontological, archaeological and geological survey in the Awash River Valley, Ethiopia, members of the Rift Valley Research Mission in Ethiopia (RVRME) recovered much of the facial skeleton and neurocranium of an early hominid. The specimen, which we describe here, is one of the best preserved and most complete of its kind yet discovered in Africa and is unique in its close association with stone tools.

The site of the discovery, Bodo D'Ar, is situated in north-western Hararge Province (Fig. 1). Hominid material was first discovered at the site in autumn 1976. Field work completed by March 1978 has produced abundant faunal and archaeological material associated with the hominid specimen. The fauna and associated artefacts are derived from layer B of the Upper Bodo Beds (J.E.K. *et al.*, unpublished). The presence of many hippopotamus remains, including several skeletons, associated with stone tools, suggests that this was a site at which carcasses were

butchered by early hominids. Further archaeological investigations, to be carried out in the near future, should elucidate the nature of this association in more detail (W. Singleton, P. Larson, H. Mosca, F. Wendorf, unpublished). The palaeontological and archaeological evidence is consistent with a Middle Pleistocene (0.7–0.125 Myr BP) age for the hominid-bearing deposits (J.E.K. *et al.*, unpublished).

The specimen was discovered after weathering out of the surface of the Upper Bodo sand unit. This consists of 5.5–6.0 m of horizontal grey conglomerate and sand and contains many mammalian fossils and artefacts. Virtually all the artefacts are made from basalt. Two large pieces of the facial skeleton were found 11 m apart by Alemeyehu Asfaw, P. Whitehead and C. Wood. The first piece to be discovered consisted of the lower part of the facial skeleton. This fragment had been broken from the upper face along a line running across the middle of the left orbit and through the nasion. It included the facial skeleton below the midpoint of the orbits, together with most of the palate. The second fragment included the upper half of the left orbit, most of the right orbit, the anterior part of the frontal bone, and the basicranium from the pterygomaxillary fissure to the basion. As the fit of these two pieces along the line of breakage is perfect, there is no doubt as to the proper alignment of the face and the cranial vault.

Forty-six smaller skull fragments were collected from the area immediately surrounding the two major fragments in 1976 and a further 30 pieces were found by sieving the area in 1978. Of this total, 41 were subsequently pieced together and fitted to the facial-frontal fragment. All fragments are in direct contact with each other and thus we have little doubt that the shape of the reconstructed cranium accurately reflects the true contours of the cranial vault.

The specimen consists of an almost complete face and partial neurocranium and includes most of the frontal bone, both nasal bones, the left zygomatic bone except for the temporal process, the left maxilla and part of the right maxilla, the sphenoid bone and portions of the left temporal bone, left and right parietal bones, and occipital of the right side. The orbits, the nasal cavity, the anterior part of the endocranial cavity and part of the cranial base are still encrusted with a hard matrix of sandy micro-conglomerate. Many of the more delicate bones such as the lacrimals, ethmoids and lesser wing of the sphenoid are present within the matrix (Figs 2–4).

Much of the basicranium anterior to the basion is present, including the left mandibular fossa and articular eminence, the basioccipital and the petrous portion of the temporal bone. Foramina for the mandibular division of the trigeminal nerve (foramen ovale), the middle meningeal artery (foramen spinosum) and the internal carotid artery (carotid canal) are visible. About two-thirds of the palate is preserved and the roots or alveoli of all the maxillary dentition, except for P⁴–M³ on the right side, are present but no crowns of teeth are preserved. Posterior to the right fourth premolar the maxilla has been broken away revealing a large, matrix-filled maxillary sinus.

The skull is large and ruggedly built and the face is notably large and robust. The nasal root is broad, the supraorbital ridges are thick and the zygomatic bone is deep and heavily constructed. The supraorbital ridges are arched and separated by a prominent glabellar region, rather than forming a continuous bony shelf; they are approximately 17–18 mm thick at the midpoint of the orbital rim, thicker than the average for the Choukoutien *Homo erectus* sample (15.5 mm) and at the high end of the range for Javan *Homo erectus* and the Solo skulls^{1,2}, but somewhat less than the Broken Hill specimen (about 21 mm). The total breadth across the supraorbital torus (139 mm) is greater than that of any of the Javan fossils (110–126 mm), being more comparable to that of the Broken Hill specimen (141 mm). The interorbital breadth at the maxillo-frontale is great (34.5 mm) and the piriform aperture broad and low. The nasals are moderately prominent in norma lateralis, being comparable to those of the Broken Hill specimen.

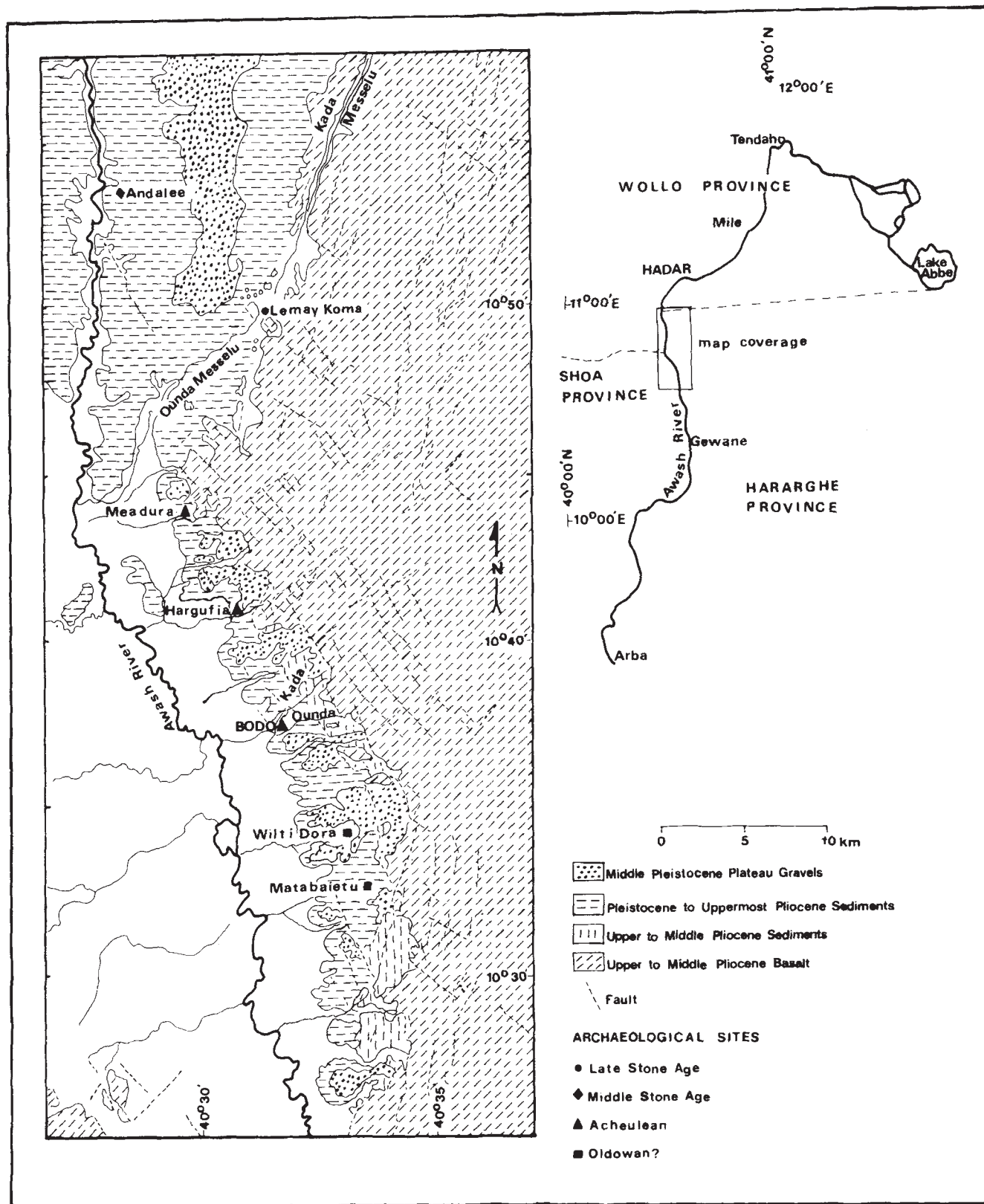


Fig. 1 Map of Middle Awash Valley (by E. B. Oswald)

One of the most striking features of the face is the depth and robusticity of the zygomatic bone. Its depth at the zygomatico-maxillary suture (40 mm) far exceeds that of the Broken Hill skull (27 mm) or the classic Neanderthal of La Chapelle-aux-Saints (about 24 mm, measured on a cast). As in the Broken Hill skull, the zygomatic flares more directly laterally than is characteristic of many classic Neanderthal skulls³. Facial breadth in the Bodo hominid (158 mm) is almost identical to that recorded for

Sangiran 17 and considerably greater than the comparable dimension in the Broken Hill specimen (about 135 mm). The nasion-prosthion length (88 mm) is close to that of the Broken Hill skull (94 mm) and the newly described *Homo erectus* (KNM-ER 3733) from Koobi Fora⁴.

The palate is both broad and deep (about 46 mm wide at M²). Perhaps because the maxillary sinus is extensive, the canine fossa is absent in the Bodo skull as in most crania of *Homo*

erectus and archaic *Homo sapiens*. As might be expected in a specimen with such heavy masticatory buttressing in the face, the articular eminence is thick. However, unlike that of most *Homo erectus* specimens and Olduvai hominid 9, the mandibular fossa is not notably deep or narrow, resembling instead that of the Broken Hill cranium⁵.

In *norma lateralis*, the reconstructed cranium is long and low. The frontal slopes gently up from the supraorbital ridges to the bregma, resulting in a low receding forehead. The glabella-bregma chord of 126 mm exceeds that of any Asian *Homo erectus* (88–112 mm) and is close to the value seen in the Broken Hill skull (122 mm). There are marked similarities between the Bodo and Broken Hill specimens in the morphology of the frontal region. Both differ from European Neanderthals as well as from *Homo erectus* from Choukoutien and Koobi Fora in lacking a supraorbital groove (ophryonic groove) backed by a more bulbous frontal region^{1,4,6}. In this respect, the Bodo skull is reminiscent of Sangiran 17, Arago, and the Petralona skull^{7–11}.



Fig. 2 Frontal view of Bodo hominid (*norma frontalis*) (scale in cm).

The temporal fossae are well enough preserved to indicate that postorbital constriction is less pronounced than in *Homo erectus*. The minimum postorbital breadth in the Bodo cranium is 114 mm but only 83–106 mm in Asian *Homo erectus* and about 102 mm in Olduvai hominid 9. In this respect, the Broken Hill cranium (105 mm) is closer to *Homo erectus* than to the Bodo skull.

As in most *Homo erectus* and many archaic *Homo sapiens* skulls there is a slight but distinct keeling of the bone along the mid-sagittal line, especially in the region of the bregma. This keel results in shallow parasagittal depressions on either side of the midline.

The greatest breadth of the cranium is undoubtedly near the asterion. In *norma verticalis* the skull seems to be somewhat

Fig. 3 Lateral view of Bodo hominid (*norma lateralis*) (scale in cm).

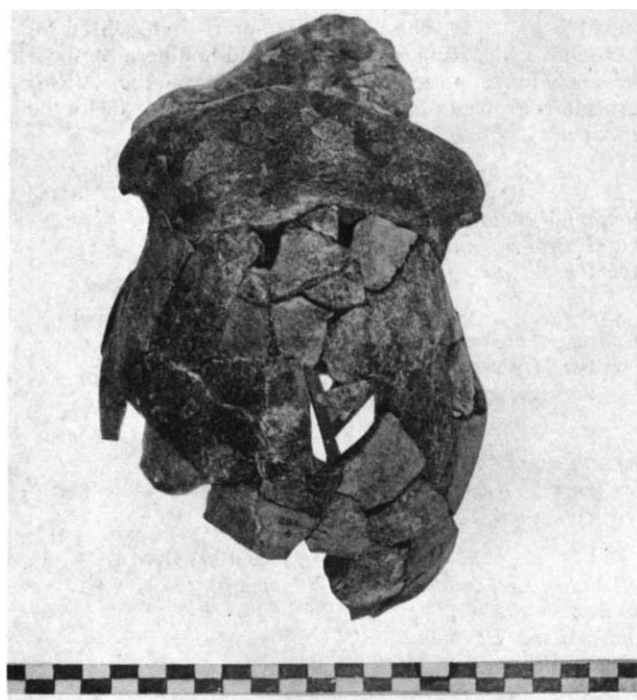


Fig. 4 Top view of Bodo hominid (*norma verticalis*) (scale in cm).

piriform in shape, broadening posteriorly. The temporal lines are clearly visible but not prominent and at the level of the bregma are about 49 mm from the midline.

One of the most impressive features of the cranial vault is the thickness of the bones. The thickness at the bregma is about 13 mm, near the pterion approximately 10 mm, and the thickness of the frontal midway between the bregma and glabella about 9 mm. The thickness at the bregma is greater than that of any Asian *Homo erectus* reported by Weidenreich, which range between 7 and 10 mm (refs 1, 12). The Broken Hill cranium, too, is apparently thinner at each comparable point.

In overall appearance, the Bodo skull is most similar to the Broken Hill and Petralona skulls. Recent reassessment of palaeontological and radiometric evidence from important Stone Age sites in southern Africa suggests that the Broken Hill specimen dates from the latter part of the Middle Pleistocene, possibly more than 0.125 Myr BP¹³. Certainly, both the Bodo and Broken Hill skulls have a more 'archaic' morphology than the undoubted *Homo sapiens* skulls from the Kibish Formation in Ethiopia¹⁴. The latter have been tentatively dated by Th/U at about 0.130 Myr BP¹⁵. On the other hand, both are less archaic than Asian *Homo erectus* or Olduvai hominid 9. The principal importance of the Bodo skull is that for the first time in Africa a well-preserved skull of this morphological grade has been derived from a context with excellent stratigraphical, palaeontological and archaeological control.

The *Homo erectus*–*Homo sapiens* transition in human evolution, and geographical variability in archaic *Homo sapiens*, is still poorly documented in Africa. The discovery at Bodo should help to elucidate this important stage in human evolution in Africa and aid the interpretation of similar but less well documented specimens such as those found at Broken Hill and Saldanha.

We thank the Ethiopian Ministry of Culture, the Ethiopian National Museum and Addis Ababa University for facilities and permission to work on the material in their care, Dr C. Stringer for allowing us to make observations on the Broken Hill cranium and Mr R. Leakey for permission for observations on material in the Kenya National Museum, and the Spencer Foundation, Fund for Overseas Research Grants and Education, Wenner-Gren Foundation for Anthropological Research, L. S. B.

Leakey Foundation, Boise Fund, Explorers Club, USIH, New York University, Rutgers University and Southern Methodist University for support, and all our colleagues in the RVRME, particularly Professor Fred Wendorf and E. B. Oswald for their support.

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Received 22 May; accepted 4 September 1978.

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Growth limitation of a coastal diatom by low zinc ion activity

THE total zinc concentration in unpolluted marine waters has been reported to be in the 10^{-10} M range¹. Such low concentration of an essential micronutrient suggests that the growth of some phytoplankton may be zinc limited. Of the trace metals necessary for phytoplankton growth, only iron has been considered a potential limiting micronutrient in the marine environment^{2,3}. On the basis of laboratory work which focused mostly on copper, it is well known that the toxicity of a trace metal depends on its chemical speciation and can be related uniquely to its free ion activity^{4,5}. However, it has not been established unequivocally that the availability of some metals may also be controlled by their free ion activities and may thus be depressed by organic complexation. Here we report laboratory experiments demonstrating that the zinc ion activity (rather than the total zinc concentration) can limit the growth rate of a coastal diatom *Thalassiosira weissflogii* (Grun. = *T. fluviatilis* Hust.) and that the limitation occurs at zinc ion activities which would be present in unpolluted seawater if any organic complexation of zinc were taking place.

Together with iron and copper, zinc is one of the trace metals most widely required for biological processes. It is generally considered essential for both plant and animal growth and it is a cofactor of several enzyme systems, such as alcohol dehydrogenase, carbonic anhydrase and carboxypeptidase⁶. In algae, most of the detailed work on physiological responses to zinc deficiency has been carried out with *Euglena*⁷. For example, evidence for a zinc-dependent lactate dehydrogenase has been

Table 1 Total zinc concentrations necessary for given zinc ion activities at the various (EDTA)_T levels in the media

$-\log [\text{Zn}^{2+}]$	5.0	$-\log (\text{EDTA})_T$ 4.3	4.0
12.3	8.9	—	—
11.9	—	7.8	7.5
11.7	8.3	7.6	7.3
11.5	8.1	7.4	7.1
11.2	7.8	7.1	6.8
10.9	7.5	6.8	6.5

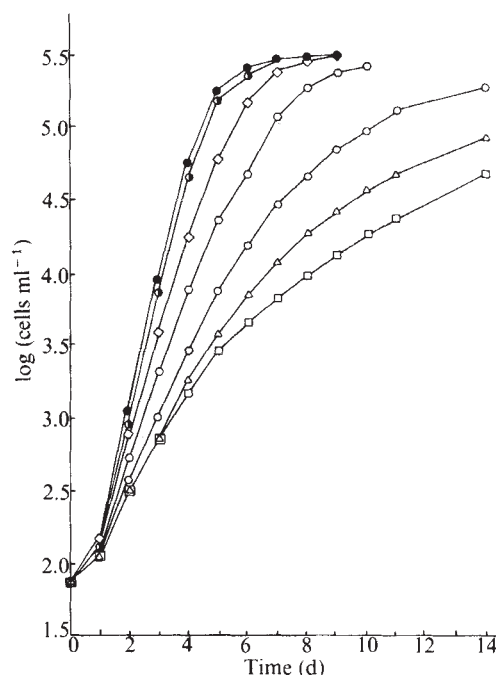
obtained in heterotrophic growth conditions⁸. Also, in autotrophic cultures, a linear relationship has been demonstrated between growth rate and zinc quota⁹, and a decrease in RNA and protein synthesis has been related to zinc deficiency¹⁰.

In our study, cultures of *T. weissflogii* (clone Actin) from the collection at the Woods Hole Oceanographic Institution were grown in 500-ml polycarbonate flasks under continuous light (Sylvania 'cool white', 10 klux) at 20 °C. Culture media followed the basic recipe of the medium Aquil¹¹ designed to permit precise control of trace metal speciation. Eleven zinc ion activities were obtained, each in three different ways, by 33 combinations of zinc and EDTA concentrations, each of these in duplicate. Metal activities were calculated with the chemical equilibrium program MINEQL¹². To avoid possible ambiguities due to the effect of EDTA on other trace metals, the activities of copper and manganese were kept constant in all experiments by appropriate modification of their total concentrations for each EDTA concentration ($[\text{Cu}^{2+}] = 10^{-14.4}$ M; $[\text{Mn}^{2+}] = 10^{-8.3}$ M). The total iron content was kept constant at 10^{-7} M, a concentration that preliminary experiments had shown did not limit the growth rate of *T. weissflogii* in the conditions of this study.

Low-density inocula were used to allow at least three orders of magnitude increase in cell density (monitored on a Coulter counter) over the growth period. At stationary phase, the maximum cell density was $\sim 2 \times 10^5$ cells ml⁻¹ and the cells were found to be silicate limited at this point.

Figure 1 shows a typical set of growth curves for one EDTA concentration (10^{-5} M) and various zinc concentrations (and

Fig. 1 Time course of cell density for various zinc additions. (EDTA)_T = 10^{-5} M. $-\log (\text{Zn})_T$: □, control; △, 9.8; ○, 8.9; ◇, 8.3; ◇, 8.1; ●, 7.8; ●, 7.5.



corresponding activities). Two other sets of growth experiments (EDTA = $10^{-4.3}$ M and $10^{-4.0}$ M) behaved qualitatively in the same manner. The growth rate of *T. weissflogii* increased systematically with zinc concentration to a maximum value. With no addition of zinc to the medium, the lowest growth rate was obtained. However, the cells in this control kept growing throughout the experiment. By comparison with the low zinc addition cultures, this result is interpreted most simply as a

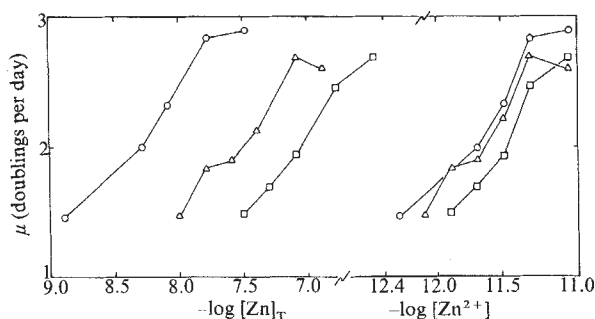


Fig. 2 Growth rate plotted against the total zinc concentration and the zinc ion activity. The relationship between total zinc and zinc activity in the media is shown in Table 1. Although no experiment has been carried out, in this work or any other, in which the free ion activity may be distinguished from the free ion concentration—which for divalent ions is greater by a constant factor of approximately 4.3 in seawater—our results are reported in terms of activity because this is the thermodynamic quantity which determines the extent of the reaction of the metal with ligands including those on or in the phytoplankton cells. ○, 10^{-5} M EDTA; △, $10^{-4.3}$ M EDTA; □, $10^{-4.0}$ M EDTA.

background zinc contamination of the order of 10^{-10} M. Growth rates for the low zinc additions are difficult to interpret because they depend on the cells' past history, on variable zinc contamination and on the depletion of zinc from the medium as the cells grow. To simplify the discussion, only the results for the cultures with the higher zinc concentrations (corresponding to activities $> 10^{-12}$ M) are thus analysed. For comparison of experiments, the exponential growth rate determined between days 1 and 3 is taken as representative of each culture.

The data from all three sets of experiments are compared in Fig. 2 and Table 1. On the left, where the growth rate (μ) is plotted against the total zinc concentration ($-\log [Zn]_T$), it can be seen that EDTA clearly influences the growth response of *T. weissflogii* with growth rates decreasing with increasing EDTA concentrations. On the right where the growth rate is plotted against the calculated free zinc ion activity ($-\log [Zn^{2+}]$), all three sets of experiments show the same dependence of μ on $[Zn^{2+}]$, thus establishing that the zinc ion activity rather than total zinc concentration determines the growth rate of *T. weissflogii* in zinc-limiting conditions. The onset of limitation is around $[Zn^{2+}] = 10^{-11}$ M. Above this activity, cultures grow at the maximum growth rate of ~ 2.8 doublings per day (as established in independent experiments).

An intriguing consequence of this result relates to the design of artificial culturing media. EDTA (or sometimes other chelators) is added to many recipes in relatively high concentrations to prevent iron precipitation and/or reduce the toxicity of various trace metals, particularly copper. As a result, the zinc ion activity in these media can be low. For example, the media WC (ref. 13), f/2 (ref. 14) and Aquil¹¹ have computed zinc ion activities of $10^{-12.2}$ M, $10^{-10.7}$ M and $10^{-11.5}$ M, respectively. It thus seems that zinc might have been made inadvertently limiting to at least some phytoplankters in these recipes.

Like copper, zinc is a necessary element that becomes rapidly toxic to algae as its activity is increased. According to the literature on zinc toxicity to phytoplankton, various species seem to have widely different sensitivities and some may be conditioned by long-term exposure¹⁵. Toxicity of zinc ion to *T. weissflogii* is observed in the range $[Zn^{2+}] = 10^{-9}$ – 10^{-8} M (D. J. Hughes personal communication). Thus, the range of zinc ion

activity over which *T. weissflogii* grows at its maximum rate spans only some two orders of magnitude (10^{-9} – 10^{-11} M). The natural zinc ion activity in marine waters ($\sim 10^{-10.5}$, as Zn^{2+} is the calculated major inorganic species of zinc in seawater) is somewhere at the low end of that range. If any organic complexation of zinc were to take place in an unpolluted coastal water, the growth of *T. weissflogii* could easily be limited by zinc deficiency. In contrast, in a polluted situation where the zinc concentration can attain 100–200 times its natural value, the growth of *T. weissflogii* could be impaired by zinc toxicity.

The present study illustrates the potential importance and the complexity of trace metal–phytoplankton interactions in natural waters; metals can be limiting or toxic depending not only on their concentrations and the algal species considered, but also on their chemical speciation. Zinc ion activity may be one of those elusive factors which control the quality of seawater to support phytoplankton growth.

This work was supported by USNF grants OCE-7709000 and OCE-7610876. We thank Dr S. W. Chisholm for useful comments.

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Rodent pollination in southern African *Protea* spp.

THE pollination of some Australian Proteaceae by rodent-like marsupials was suggested about 40 years ago¹. This subject has been studied little and until quite recently current summaries of pollination biology gave little attention to pollination by nonflying mammals^{2,3}. We present here evidence that rodents regularly pollinate two Cape *Protea* spp., *Protea amplexicaulis* and *P. humiflora*, and that the flowers are specifically adapted for such pollinators.

These *Protea* spp. are low, spreading shrubs which bear their flowering heads near ground level (geoflorous), largely hidden from external view by the overlying foliage (cryptic)⁴. Thus they are distinct from the horticulturally well known bird-pollinated *Protea* spp., which are tall shrubs or small trees with large, conspicuous, brightly coloured heads borne terminally on branches several metres above the ground.

The studies were conducted between mid-August and mid-September on altitudinally discrete populations of *P. amplexicaulis* and *P. humiflora* on Jonas Kop in the Riviersonderend-berge approximately 100 km east of Cape Town. The results of

snap (kill) trapping around the flowering heads of *P. amplexicaulis* and *P. humiflora* showed that 100% of the animals carried an exclusively *Protea* pollen load on their snouts, but only about 50% of the live trapped animals captured in Sherman live traps carried *Protea* pollen (Table 1). After foraging the mice wet-reen their snouts with the forepaws and quickly remove much of the pollen which is presumably ingested. Snap trapped animals are killed quickly and their pollen retained. Live trapped animals remained in the traps overnight (up to 14 h), allowing them ample time to preen off pollen.

The capture of shrews carrying *Protea* pollen probably reflects only the strength of the nectar as a broad-spectrum mammal attractant, as their snouts appear too small to effect pollination consistently. Captive namaqua rock mice (*Aethomys*), verreaux' mice (*Praomys*), and Cape striped field mice (*Rhabdomys*) readily forage from flowering heads but Cape striped field mice show both weaker and more destructive foraging activity than the other species. When fasted for 24 h verreaux' mice will feed on nectar for up to 15 min. Sixteen-millimetre movies of captive mice lapping nectar from the heads of both species were taken between 20.00 and 23.00 when these nocturnal animals are active. Cinephotography demonstrates convincingly and dramatically the process of rodent pollination.

Both the heads and individual flowers of *P. amplexicaulis* and *P. humiflora* exhibit remarkably similar structural features which adapt them to rodent pollination. The bowl-shaped heads (up to 12 cm wide) are borne on short, stout peduncles which are capable of supporting the weight of these relatively heavy animals (20–70 g). The heads are whitish on the inside which contrasts with the surrounding dark, reddish bracts (presumably an adaptation for nocturnal pollinators). The style bases block effective access to the nectar if approached from the perimeter of the head and pollinators must forage outward along a radius from the centre of the head (Fig. 1a). The nectar accumulates in a bowl-like structure formed from the petals and is positioned about 10 mm below and slightly behind the stigma. The styles of individual flowers are first arched slightly backward and then strongly inflexed at least 45° (Fig. 1b). They are fibrous (almost wire-like), but highly resilient which allows forceful manipulation by rodent pollinators without damage. Pollen is dispersed from the apical region of the style known as the pollen presenter and the stigmatic region is reduced to a minute slit at the extreme apex of the style immediately in front of the pollen presenter (Fig. 1b). The style thus functions as both pollen disperser and receptor. It is not known if these species are capable of self pollination, but protandry and self incompatibility are acknowledged methods of preventing self fertilisation in other Proteaceae^{5,6}.

The critical pollinatory features of the flowers (nectar, pollen, stigma) and rodent snout produce a typical complementary

flower–pollinator 'fit' (Fig. 1b) and large amounts of the sticky pollen are deposited on the snouts of foraging animals (Fig. 1a). Simultaneously, pollen is forced into the minute stigmatic slit which may require a large, relatively powerful pollinator. Proteaceae pollinated by insects require only light physical application of pollen to the stigmatic surface to effect pollination⁷.

The heads produce a distinctive 'yeasty' odour (presumably the initial attracting mechanism) and nectar in millilitre quantities (*P. amplexicaulis*, mean = 2.4 ml; *P. humiflora*, mean = 1.7 ml). The individual flowers in the former produce an average of 12.4 µl and the latter 9.8 µl of nectar. Nocturnal nectar secretion is suspected but data on rhythmicity are not yet available and average amounts of nectar can be extracted from the heads at any time. *Protea humiflora* produces about 57 heads per plant annually and each head bears about 300 flowers. The plants are relatively common in the study area and this nectar must be an important community food resource for rodents, especially as flowering in these proteas occurs in late winter, typically a low point in rodent food cycles.

The following basic features characterise mouse-pollinated *Protea* spp.: (1) a distinctive 'yeasty' odour; (2) copious nectar production (2–3 ml per head); (3) nocturnal pollinator activity; (4) inflexed, relatively short (about 30 mm), wiry, flexible styles; (5) bowl-shaped heads with whitish interiors surrounded by dark, reddish bracts; and (6) cryptic, geoflorous positioning of the heads. The latter feature, although well developed in *P. amplexicaulis* and *P. humiflora*, may not occur in all rodent-pollinated species. Applying these criteria to *Protea* as a whole, rodent pollination is predicted in about 35 species and multiple independent evolutionary origins are suggested.

In the Australian proteaceous genus *Banksia* recent evidence is available for pollination by the indigenous southern bush rat (*Rattus fuscipes*)⁸. These banksias also possess the floral characteristics of the Cape rodent-pollinated *Protea* spp., but the inflorescences (spikes) are not necessarily cryptic or geoflorous. These data demonstrate the occurrence of strong convergent floral evolution between Cape and Australian Proteaceae for pollination by nonflying mammals and support the hypothesis that they compose a distinct floral class (therophily)^{4,9}.

Because rodent pollinated proteas do not flower throughout the year co-adaptation is impossible. Pollinating rodents are apparently generalist feeders enticed to the plants by the rich nectar reward. In Australia, however, therophilous plants flower throughout the year and some rodent-like marsupials are co-adapted with this floral class, for example, the honey possum (*Tarsipes spencerae*)⁴.

Recently, small arboreal primates have been reported to pollinate, or at least take nectar regularly, from certain flowers in both the Old and New World tropics^{10–14}. Whether another

Table 1 Nonflying mammals trapped near flowering heads of *Protea amplexicaulis* and *P. humiflora*

Animals	Trapping method	<i>P. amplexicaulis</i> No. animals caught	No. with pollen	Trapping method	<i>P. humiflora</i> No. animals caught	No. with pollen
Rodents (Muridae)						
<i>Aethomys namaquensis</i>	Snap	1	1	Snap	8	8
	Live	11	4			
<i>Praomys verreauxi</i>	Live	3	3	Snap	1	1
<i>Rhabdomys pumilio</i>	Snap	7	7	Snap	1	1
	Live	1	0			
<i>Acomys subspinosus</i>	Snap	3	3	Snap	2	2
<i>Mus minutoides</i>				Snap	1	1
Rodents (Gliridae)						
<i>Graphiurus ocularis</i>	Live	1	1			
Shrews (Soricidae)						
<i>Crocidura</i> sp.	Snap	2	2			
Shrews (Macroscelididae)						
<i>Elephantulus</i> sp.				Live	1	0

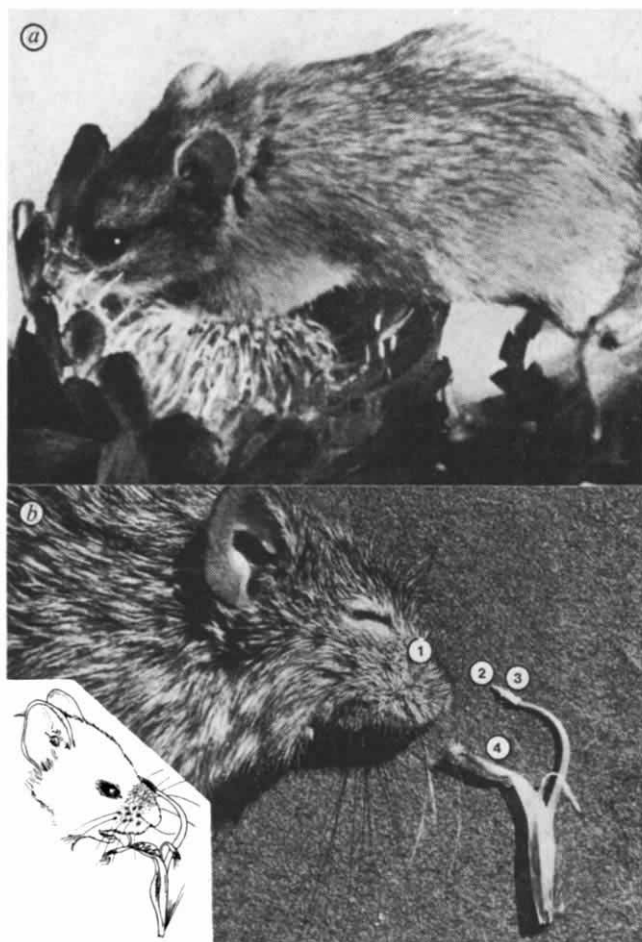


Fig. 1 *a*, Captive *Aethomys namaquensis* foraging for nectar on a flowering head of *Protea amplexicaulis*. The anterior half of the snout has accumulated a dense pollen load which appears whitish in the photograph. *b*, *Rhabdomys pumilio* and an individual flower of *Protea amplexicaulis*. 1, Area of pollen deposition; 2, location of the stigmatic slit; 3, pollen presenter; 4, nectar accumulation 'bowl'. Insert, diagrammatic representation of flower-pollinator 'fit' when the pollinator laps nectar.

floral class has evolved for pollination by this group of animals is at present uncertain.

This study was supported by NSF grant DEB 75-21170. We thank D. Alstad, S. Hartman and P. Smits for photographic and illustrative preparations, F. du Plessis for access to the study area, B. Bayer, J. Jarvis, W. Strickland, I. Walters and P. van Wyk for assistance.

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Received 12 June; accepted 4 September 1978.

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A winged élite in a subcortical beetle as a model for a prototermit

TRUE sociality has evolved in only two insect orders—the Hymenoptera (ants, bees and wasps) and the Isoptera (termites). The hymenopterans seem to have achieved social organisation on at least 11 independent occasions¹; this seems to have been facilitated by their haplodiploid sex determination which predisposes them to evolution of a caste system based on altruism². Among the termites, fundamental similarities of social adaptation and organisation indicate a monophyletic origin; but in the absence of haplodiploidy, the conditions in which eusociality has arisen remain obscure. I report here a reproductive polymorphism in tiny feather-winged beetles (family Ptiliidae) living in dead wood, in which winged females are super-reproductive. The parallel with the winged reproductives of termite societies is striking and to my knowledge has not previously been observed in a non-social insect. The selection pressures that resulted in this convergent evolution may reflect those with which the prototermit had to contend during early socialising stages. This may be a better model for the prototermit than the currently favoured blattoid *Cryptocercus*¹. The model is, of course, entirely one of an analogy based on convergence; there is no suggestion that there is any phylogenetic link.

The feather-winged beetles *Ptinella aptera* (Guérin-Meneville) and *P. errabunda* Johnson, belong to a taxonomically varied group of insects found in the subcortical habitat of rotting wood. The distribution and durability of this habitat is irregular and unpredictable³ and its supply of resources, although rich, is exhaustible. Thus, these insects depend for their perpetuation on a high degree of dispersal. Many of them show striking superficial similarity to the worker castes of termites and dispersal is, as in termites, often achieved through wing polymorphism⁴.

In *P. aptera* and *P. errabunda* wing polymorphism is extreme. The apterous morph most commonly found under bark is wingless and eyeless and has a poorly sclerotised cuticle—characteristics of termite workers. The less common alate morph

Table 1 Oviposition in alate and apterous *Ptinella*

		Total oviposition (eggs per female)	Oviposition rate (eggs per female per day)
<i>P. aptera</i>			
alate	(15)	56.86 ± 4.56	3.13 ± 0.12
apterous	(20)	29.35 ± 4.07	1.51 ± 0.15
<i>P. errabunda</i>			
alate	(6)	31.16 ± 6.11	1.12 ± 0.10
apterous	(20)	17.55 ± 2.02	0.90 ± 0.04

Adult females were maintained individually in cultures at 20 °C and approximately 100% relative humidity. *P. errabunda* is thelytokous but *P. aptera* is bisexual, and each female was accompanied by a male. Cultures were checked every 2 d and the numbers of eggs recorded. Cultures were terminated on death of the female. Males dying or found to be infertile were replaced. The data are presented as the mean ± s.e.m. Numbers of females are given in parentheses. Statistical analyses were made using Student's *t* test. Differences between alatae and apterae are significant at $P < 0.001$ for *P. aptera* and $P < 0.05$ for *P. errabunda*.

(which rarely exceeds 5% in natural populations^{5,6}) has well developed compound eyes, a heavily sclerotised cuticle and the characteristically elaborate feather wings. These morphs are similar to termites even in their reproductive biology: alatae are more fecund than apterae and have larger spermathecae and thus a greater capacity for sperm storage.

I investigated the reproduction of *Ptinella* in laboratory cultures based on those described by Goto⁷ for Collembola. Fecundity schedules were constructed for alate and apterous morphs of both species. As Table 1 shows, total oviposition and rate of oviposition were significantly greater for alatae than for apterae, although the difference was less marked in *P. errabunda* (*P. aptera*, $P < 0.001$; *P. errabunda*, $P < 0.05$).

Oviposition alone is not a reliable indicator of reproductive success. The contribution that an individual makes to succeeding generations depends on overall fertility, longevity and rate of development. These factors are taken into account in the estimates of the three population statistics (Table 2) computed for apterous and alate *Ptinella*. The estimates of rate of increase and net reproductive rate of *P. aptera* alatae are approximately twice as great as those for apterae. The mean generation times are similar showing that the greater power of alatae to increase is achieved by an increase in reproductive rate, not by a reduction in generation time. The difference between morphs is less pronounced in *P. errabunda*, but this species is thelytokous (no males have been found) and thus less termite-like than *P. aptera*.

The larger spermathecae of alatae (which have a parallel in some social hymenopterans) suggest that alate *Ptinella* are super-reproductive in natural populations. I found that the volume of the spermatheca of *P. aptera* was nearly twice as large in alatae as in apterae (means $\pm$ s.e.m. are: $9.35 \times 10^{-5} \pm 0.27 \times 10^{-5} \text{ mm}^3$ and $5.84 \times 10^{-5} \pm 0.14 \times 10^{-5} \text{ mm}^3$; $t = 11.39$, $P < 0.001$). Figure 1 shows that this difference in spermatheca size does not merely reflect a difference in body size between the two morphs.

The increased capacity to store sperm associated with increased fecundity, is of great selective value to a dispersive morph of a species that requires repeated insemination, as *Ptinella* does⁵. Nevertheless, super-fecundity of an alate in the presence of a numerically predominant, but reproductively inferior, apterous morph is paradoxical. Apterous individuals do not have to invest energy in wing and eye development and

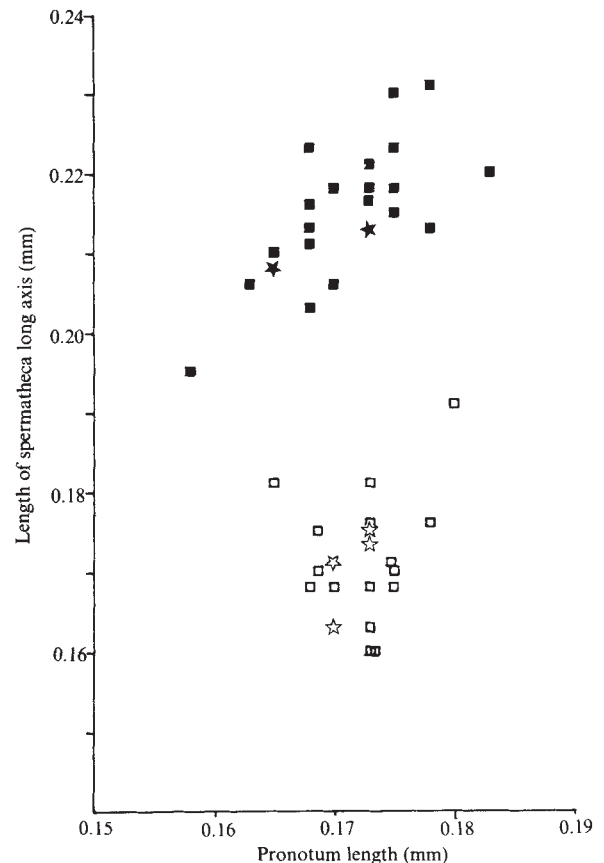


Fig. 1 The relationship between spermatheca size, body size and morph in *P. aptera*. Measurements were made on a randomly selected sample of 24 alate (■) and 24 apterous (□) beetles mounted in poly-vinyl lactophenol. The length of the spermatheca long axis was found to give a reliable reflection of volume ($r = 0.854$; $P < 0.001$) and was used to represent spermatheca size. The distance between anterior and posterior margins of the pronotum was measured as an independent variable of body size. There is no correlation between length of long axis of spermatheca and pronotum length ($r = -0.022$; $P > 0.10$). ★ and ☆ represent two points that coincide.

Table 2 Estimates of population statistics for alate and apterous *Ptinella*

		Rate of increase (r) (per female per day)	Net reproductive rate (R_0) (females per female per generation)	Mean length of generation (G) (d)
<i>P. aptera</i>				
alate	(15)	0.049 ± 0.001	13.87 ± 1.21	55.1 ± 1.3
apterous	(20)	0.026 ± 0.002	5.20 ± 0.72	57.2 ± 1.7
<i>P. errabunda</i>				
alate	(6)	0.033 ± 0.003	11.66 ± 2.29	64.7 ± 2.7
apterous	(20)	0.025 ± 0.002	6.24 ± 0.72	67.8 ± 1.9

Population statistics were computed from fecundity schedules for individual females and mean values for mortality and duration of pre-reproductive stages at 20 °C (program: W. D. Hamilton, unpublished) where $\sum_0^\infty e^{-rx} l_x m_x = 1$; $R_0 = \sum_0^\infty l_x m_x$; $G = \sum_0^\infty x l_x m_x / \sum_0^\infty l_x m_x$ and l_x = no. of survivors at the start of age interval x , m_x = no. of female offspring at the start of age interval x . In practice, morphs were indistinguishable in immature stages. As alatae tend to produce a greater proportion of winged offspring than apterae⁵, pre-reproductive mortality and development rate for *P. aptera* alatae were based on rearings of progeny of alate parents. The only obvious difference from offspring of apterae was a bias in sex ratio in favour of females⁵. The data are presented as the mean $\pm$ s.e.m. (the latter only reflects variation in mortality and fertility of adult females). Numbers of females are given in parentheses.

would be expected to achieve at least as great a reproductive output as alatae. This seems to be so in all non-social, wing polymorphic insects for which comparable data exist, loss or reduction of wings often being associated with a tendency towards increased fecundity⁸. The alate *Ptinella* may represent the beginnings of a winged, reproductive elite of the kind that has evolved to its ultimate expression in the termites and ants. The implied reproductive altruism is a fundamental characteristic of insect societies.

Can *Ptinella* then, provide any clues concerning the prototermite? Primitive cockroaches are considered to be the progenitors of the first termites⁹, of which the communal blattoid *Cryptocercus* is popularly supposed to provide a living model. It is certainly very similar in details of morphology and physiology to the relatively 'primitive' *Mastotermes*, particularly in its ability to digest cellulose with the aid of symbiotic Protozoa. However, *Cryptocercus* shows no sign of the polymorphism that is an intrinsic feature of termite society. In this respect *P. aptera* is certainly more termitoid. Its convergent characteristics are made more impressive by its phylogenetic remoteness from the termites. Such convergence suggests that in crucial stages of socialisation the prototermite may, like *Ptinella*, have been subject to the particular selection pressures of the subcortical habitat.

I suggest therefore, that *P. aptera* may prove to be a realistic model on which to base speculation concerning the bionomics of

the prototermite and the conditions in which it was living when it embarked on the evolutionary pathway to eusociality.

I thank Dr W. D. Hamilton for advice and encouragement. This work was supported by a NERC studentship. I thank Professor T. R. E. Southwood for use of facilities at Imperial College Field Station, Silwood Park, where the work was carried out.

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Received 21 June; accepted 4 August 1978.

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Reorganisation of spinal cord sensory map after peripheral nerve injury

It has generally been assumed that the connections of spinal cord cells are laid down during development and then remain stable throughout adult life. However, it has been shown that dorsal horn cells can be excited in a novel fashion by afferents over distant intact dorsal roots if section of nearby dorsal roots results in the degeneration of the afferent sensory fibres which normally activate the cells¹. Here we show that this plasticity of afferent connection can be provoked by section of peripheral nerves where there is no gross anatomical change of the central terminals of the sectioned fibres and yet the cells on which the cut nerves end begin to respond to the nearest intact nerves.

Our experiment is based on the fact that the dorsal horn of lumbar segments 6 and 7 contains somatotopically organised cells with the foot and toes represented medially and the upper and lower leg laterally^{2,3}. The medial dorsal horn cells can therefore be peripherally deafferented by transection of the nerves to the foot, the sciatic and saphenous nerves.

Using adult cats weighing more than 2 kg we severed the sciatic and saphenous nerves in the mid-thigh and ligated the proximal ends tightly. This produces a complete and permanent anaesthesia of the foot and toes as judged by absence of the flexor reflex from the foot and failure to discover any cord cells driven by mechanical or electrical stimulation below the ankle. At intervals after the nerve lesion the peripherally deafferented dorsal horn was examined in acute preparations for cells responding to cutaneous stimulation of the leg. In these acute preparations the cats were anaesthetised with ether and decerebrated at the mid-collicular level. Ether was then discontinued, paralysis induced with Flaxedil, the spinal cord cut at L1 to maximise excitability and the lumbar enlargement exposed and placed under warmed (37°C) mineral oil. Physiological condition was maintained in the usual manner; cord circulation was brisk and blood pressure remained above 80 mm Hg. Recordings were made with 1–3-M Ω KCl-filled glass electrodes inserted and located by methods previously described^{3–6}. In each animal, the dorsal horn was examined to a depth of 2 mm below the surface with a series of parallel tracks separated by 100 μ m beginning at the mid-line and moving laterally to beyond the dorsal root entry zone (REZ).

Graded mechanical stimuli used to characterise receptive fields (RFs) were: rapid movement of hairs with a no. 8 camel hair brush, touching the skin's surface with a blunt probe (4 mm²

tip, force 1–20 g), and pinching with toothed forceps at levels considered mildly painful by human observers.

Sixteen maps were completed in acutely deafferented dorsal horns of 12 cats, with a total of 156 search tracks at or medial to the REZ. Mechanical and electrical stimuli were applied to the skin continuously throughout the search. In all animals, a cluster of cells was located in the dorsolateral grey matter with RFs on the upper and lower leg (Fig. 1, right side). These cells had response characteristics and latencies to electrical stimulation of their RFs apparently identical to those described in the intact cat^{3,5,6}. As penetrations moved more than 200 μ m medial to the REZ, however, large numbers of cells were encountered which had continued activity but which failed to respond to peripheral stimulation. In this medial area of dorsal horn, we located only eight responding cells in 108 search tracks. All eight required pressure on the upper or lower leg to excite them. In the intact cat, virtually all cells in this region of dorsal horn respond to brush, touch or pressure stimuli on foot or toes^{2,3}. Evidently, the peripheral nerve section had deafferented nearly all the medial dorsal horn cells.

We allowed seven animals to survive 28, 31, 34, 54, 61, 70 and 105 d, respectively, after nerve section. In each of these chronic animals, the sciatic and saphenous nerves were acutely sectioned in the opposite leg on the day of the examination. This allowed maps to be made of one dorsal horn which had been chronically deafferented and comparison with the opposite dorsal horn which was acutely deafferented. In four of the seven, the sciatic and saphenous nerves which had been cut previously were exposed again on the experimental day and resectioned 10–20 mm proximal to the nerve end neuroma. This was done for two reasons. First, it eliminated the possibility that sprouts had grown out of the neuroma and established contacts in the upper leg. Second, it prevented the appearance of false RFs

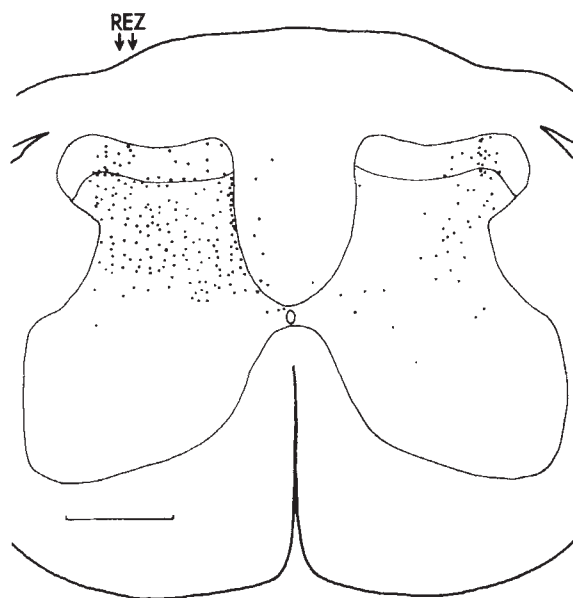


Fig. 1 Location of cells in the L6–7 segments of spinal cord that have cutaneous RFs after acute (right) and chronic (left) section of the sciatic and saphenous nerves. Dots mark all satisfactorily isolated cells encountered in 164 penetrations in 12 acutely deafferented dorsal horns and 89 penetrations in 7 chronically deafferented dorsal horns (28–105 d survival). The arrows (upper left) mark the approximate location of the root entry zone (REZ) as seen from above. Its exact location varies somewhat between cats. Search penetrations began near the midline and continued to about the REZ but not much beyond it. The lack of cells in the far lateral edge of dorsal horn therefore does not indicate the absence of proximal field cells there⁴. Units within the dorsal column are correctly located and probably represent stray secondary neurones or their processes. Scale bar, 1 mm.

in the upper leg due to mechanical spread of stimuli directly to the neuroma, which we know becomes sensitive to light pressure^{7,8}.

The most obvious change in the chronically deafferented dorsal horns was that the most medial track containing responding cells had moved from about 200 μm medial to the REZ on the acutely deafferented side to the far medial edge of the dorsal horn (Fig. 1). In 69 tracks more than 200 μm medial to the REZ, 94% (65 tracks) contained cells with RFs on the upper or lower legs compared with 7% in the acutely deafferented cords. RFs from 160 cells in this region were examined; 25% of the cells were brush-sensitive, 43% were sensitive to touch but not brush, and 32% required pressure stimuli. There were, of course, no cells with RFs on the toes or foot. The size, nature and position of the RFs covered the same range as those previously described for laterally placed cells with proximal RFs in intact animals⁵ and those we observed here in the same region in the acutely deafferented dorsal horns. Latency of the fastest response to electrical stimulation applied to the RF was measured for 99 cells located at least 200 μm medial to the REZ in chronically deafferented cats. Latencies ranged from 3.0 ms to more than 15 ms (8.0 ± 3.2 ms mean $\pm$ s.d.). Those cells responding in 3–4 ms very probably have a monosynaptic contact with afferents and they made up 19% of the population. The new RFs found in the former toe-foot region were all in skin supplied by remaining intact cutaneous levels. We cannot attribute the new RFs to sprouting from the sectioned nerve into the upper or lower leg, as we acutely resectioned the experimental nerves in most cases before mapping. Pooled measurements of dorsal horn width based on cresyl violet-stained sections of all experimental animals showed that the chronically and acutely deafferented sides were symmetrical to within 2%. It is therefore highly unlikely that we could have been recording from normally connected cells which had migrated or had been displaced. We are left, then, with the most probable conclusion that cells which previously responded to foot-toe stimuli shift their RFs to more proximal loci. The shift of RFs was apparently fully established within 28 d, as we could detect no quantitative differences between the animals examined over the survival span of 28–105 d. However, in a further six animals with survival times of 6–19 d, we observed a progressive development of the picture we have presented here for the mature animals. In these short-term animals, there was a progressive movement of the lateral margin of responding cells into the medial area of deafferented cells, with the number of responding cells increasing and their threshold decreasing.

What central changes after peripheral nerve section could account for this shift? Some subtle chemical and atrophic morphological changes have been seen in the substantia gelatinosa but there is no frank degeneration of primary afferents such as occurs after section of dorsal roots, and synaptic sites are not vacated^{9–13}. We have examined in light microscopy the L6–7 cord segments of nine cats 13–105 d after chronic sciatic and saphenous nerve lesion. Virtually no residual debris was found on the experimental side in material stained with the Fink-Heimer, Fink-Schneider (normal fibre stain) or cresyl-violet methods. In contrast, a moderate amount of transganglionic degeneration can be detected by these methods in the trigeminal nucleus after 5th nerve injury^{14–16}. Furthermore, medial dorsal horn cells continue to respond briskly to stimulation of the sciatic nerve stump. We cannot, however, rule out the possibility that very small numbers of primary afferents are lost. More saliently, impulse traffic from sensory receptors and the transport of substances from the peripheral nerve terminal are arrested. Although the deafferented cord cells do not receive their normal afferent barrage, it is known that in the rat substantial numbers of cut sciatic nerve myelinated fibres generate impulses beginning 2–3 d after section, peaking at 12 d and returning to a low level at 20 d¹⁷. In the mouse, a similar phenomenon is seen beginning at 2 d, peaking at 17 and declining to a low level by 30 (J. Scadding, personal communication). Finally, section of the motor axons in the nerve causes chromatolytic and other

changes in motoneurons and this raises the possibility of retrograde transsynaptic changes in dorsal horn¹⁸.

How could these changes affect deafferented dorsal horn cells? The new RFs might be produced by new growth from nearby intact afferent terminals. Alternatively, they might be produced by an increase in the excitatory effectiveness of a previously existing but weak mono and/or polysynaptic afferent channel such as we have found on many dorsal horn cells in normal cats^{20,21}. The experiments we described here do not preferentially favour any of these possible mechanisms. It is clear, however, that peripheral nerve lesions evoke a surprising degree of plasticity of the connections responsible for forming the receptive fields of dorsal horn cells.

This work was supported by a grant from the Fritz Thyssen Stiftung. We thank M. Kaitz, D. Schonfeld, R. Govrin and I. Frank for their assistance.

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Nature of light-induced conductance changes in ventral photoreceptors of *Limulus*

THE response of invertebrate photoreceptors to light is thought to be composed of small discrete events known as 'bumps'^{1–5}. The rate of occurrence of these bumps increases and their average size decreases with increasing ambient light intensity (light adaptation)^{6–10}. The mechanisms involved in these processes are unknown. I report here an analysis of the light-induced current fluctuations in the voltage-clamped ventral photoreceptors of *Limulus*. Results seem to suggest that the conductance change of a bump is due to the opening of discrete channels which have properties similar to those of the discrete channels observed in the vertebrate neuromuscular junction^{11–14}. The data also lead to suggestions of plausible mechanisms for the generation and adaptation of bumps.

If one assumes that the steady-state response (Fig. 1) is a superposition of bumps, the shape of the bumps can be estimated from the variance (power) spectrum, and the size and rate of occurrence can be calculated from the mean (time average) and the variance^{15–17}. Figure 2 shows the variance spectra calculated from data obtained at four different light intensities.

As may be seen, these spectra can be fitted very well by an expression of the form

$$y = \frac{A}{(1 + 4\pi^2 f^2 \tau^2)^m} \quad (1)$$

where m and τ are variable parameters, and A is an arbitrary constant. In the time domain, this function, for various values of m , defines a family of curves describing the bump shape¹⁵. For a fixed value of m , the parameter τ determines the time scale of the bumps. For example, the variance spectra calculated from data obtained at log light intensities of -6.5 and -7.5 can be fitted by equation (1) with $m = 3$, $\tau = 8.44$ ms (Fig. 2) and $m = 3$, $\tau = 10.6$ ms (not shown), respectively. For $m = 3$, the bump shape inferred from the variance spectrum¹⁵ is a curve with a parabolic rise and an exponential decay. This shape is identical to that of the impulse response of a cascade of three stages of RC filter with an identical time constant (τ) for each stage²⁰. The inferred bump shape is consistent with the shape of the directly observed bumps (Fig. 1, log $I/I_0 = -7.5$, when plotted in expanded time scale). The data shown in Fig. 2 also indicate that the bump shape changes systematically from $m = 3$ at low light intensities, through $m = 2$ at intermediate light intensities to $m = 1$ at high light intensities. The background noise limits the resolution of the high-frequency components of the spectra at log intensities above -3.5 . However, if the background noise is subtracted from the measured spectra at these light intensities, the shape of the resultant spectra is usually consistent with $m = 1$. In addition to the change in m , the 'time scale' of the bumps shortens with increasing light intensity, as indicated by the relative increase in the high-frequency components of the spectra with increasing light intensity. (When the shape of the bumps changes, the time scale of the bumps is determined by both m and τ .)

From the mean, the variance and the variance spectrum, the size and rate of occurrence of the bumps are calculated using Campbell's theorem¹⁵⁻¹⁷. Figure 3 shows these values plotted as functions of light intensity, and indicates that the rate of the bumps increases proportionally to light intensity up to log intensity of -3.5 . At this light intensity ($\sim 10^5$ bumps s^{-1}), the rate departs from strict proportionality, indicating signs of saturation. The size of the bumps decreases monotonically with light intensity; between -6.5 and -3.5 log light intensities, the size of the bumps shows a negative 0.77th power dependence on light intensity. The results shown in Fig. 3 are consistent with those reported previously^{15,21}. If the driving potential is taken to be 90 mV, the conductance change due to a single bump (calculated from variance/mean, that is, the bump height^{15,17}) is 1.3×10^{-8} S at log light intensity of -7.5 , and 1.8×10^{-11} S at log light intensity of -2.5 . The conductance change due to a single bump seems to approach a lower limiting value at log light intensity of about -2.5 (ref. 15). This limiting value of the conductance change is of a similar size to those reported for a single channel in other excitable membranes ($\sim 10^{-11}$ S)^{12-14,22,23}.

Equation (1) with m of 1 is the well known lorentzian^{13,22-24}. Spectral analyses in other excitable membrane systems have yielded variance spectra which resemble the lorentzian. This particular shape of variance spectrum has been interpreted to indicate (1) that the underlying conductance channels have basically two states, a conducting and a nonconducting one, (2) that these channels fluctuate between the two states independently of one another, and (3) that the lengths of time each channel remains in the conducting state follow an exponential distribution. In the muscle endplate, single channels having these properties have been observed directly¹⁴. If the bumps are due to the opening of discrete channels in the photoreceptor membrane, which have the above properties, the results shown in Figs 2 and 3 may be interpreted in terms of the following hypothesis. At low light intensities, each bump is associated with many channels. (Remember that the bump height at -7.5 log light intensity is about 10^{-8} S which is a few orders of magnitude greater than the values reported for the conductance of a single channel displaying ion selectivity^{4,12-14,22,23}.) If the many chan-

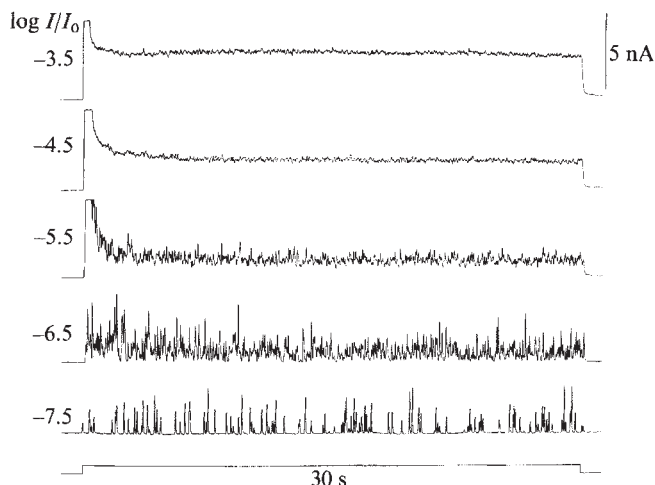


Fig. 1 Samples of responses of the ventral photoreceptor to light of different intensities under voltage clamp. The command voltage was usually set at the resting potential of the cell (-70 mV for this cell). The light source consisted of a tungsten lamp, and the unattenuated intensity (log $I/I_0 = 0$) at the level of the preparation was $\sim 10^{14}$ photons $cm^{-2} s^{-1}$ at 520 nm when measured over a 40-nm bandwidth. Stimulus patterns were generated by programming a digital timer. The stimuli were 30-s steps of light of different intensities, and were presented at 60-s intervals for log light intensities of -7.5 and -6.5 , 90-s intervals for log light intensities of -5.5 and -4.5 , and 120-s intervals for log light intensities above -4.5 . Data were recorded on tape with an FM tape recorder having a bandwidth between d.c. and 1.5 kHz. Digitisation of the data, calculation of the mean and the variance, and spectral estimation were carried out with a CDC 1700 computer. Before digitisation, the data were filtered through a 4-pole Butterworth filter having a 3-dB point at 300 Hz. The filtered data were sampled at 2 kHz. As the response to steps of light, especially to bright light, shows both a transient (peak) and a steady-state (plateau), the calculations were carried out on the data in the last 20 s of the 30-s steps to ensure that the steady-state assumption implicit in the calculations was valid. (The truncation of the peaks at high light intensities shown here was due to saturation of the recording amplifiers.) The data processing scheme was checked by electronic simulation of randomly occurring shots of known shape, size and rate of occurrence. The shape of the variance spectrum and values of the parameters calculated from the simulated noise were found to be consistent with the input.

nels associated with a single bump were opening simultaneously and closing independently, the bump shape would be an exponential function and the variance spectrum would yield m of 1 in equation (1) (ref. 13). However, if the opening of the individual channels due to a single bump is not simultaneous but somehow correlated (for example, by some cooperative mechanisms), the rising phase of a bump will not be a sharp jump but will follow some other time course.

The exact mechanisms giving rise to the observed shapes are unknown. The systematic change of the shape of the variance spectrum towards a lorentzian at high light intensities can be interpreted to indicate that the decrease in bump size with increasing light intensity occurs mainly because of a reduction in the number of channels associated with a bump. This is because, as the number of channels associated with each bump decreases, the opening of the individual channels will become less 'correlated', as the occurrence of each bump is an independent event¹⁵. When the light intensity is sufficiently high for the channels associated with the bumps to be opening and closing approximately independently of these events in other channels, the variance spectrum would approach a lorentzian (log light intensity of ≥ -4.5 , Fig. 2).

Although the above hypothesis is one of many possible interpretations of the data, it provides a good working model for the organisation of many observations. For example, assuming that

each bump at the highest light intensity studied corresponds to the opening of a single channel, the results shown in Figs 2 and 3 allow us to estimate that at 21°C an individual channel has a conductance of 18 pS and an average opening time of 18.7 ms. The time course of the falling phase of the directly observed individual bumps (Fig. 1, $\log I/I_0 = -7.5$) is found to approximate closely to an exponential. This is consistent with the present model in that when there are many channels associated with a single bump, such as at low light intensities, the opening of these channels is 'correlated' but their closing is independent. Thus, the time constant for the falling phase of a bump should give an estimation of the average opening time of the channels¹³. This time constant, measured from 15 directly observed bumps, is 14.4 ± 1.8 ms, a value some 30% smaller than that estimated above from the variance spectrum. If the average opening time is independent of bump size, this difference could mean that even at the highest light intensity studied, the individual channels are not completely independent of one another, implying in turn that the estimated values of the conductance (18 pS) and of the average opening time (18.7 ms) of a single channel may have been slightly overestimated.

The rate of occurrence of the bumps saturates at about 10^5 bumps s^{-1} when the light intensity is $\sim 10^{12}$ photons $cm^{-2} s^{-1}$. The molar extinction of rhodopsin, taken to be 40,000 (ref. 25), may be converted to a rhodopsin 'cross-section' of $\sim 10^{-16} cm^2$.

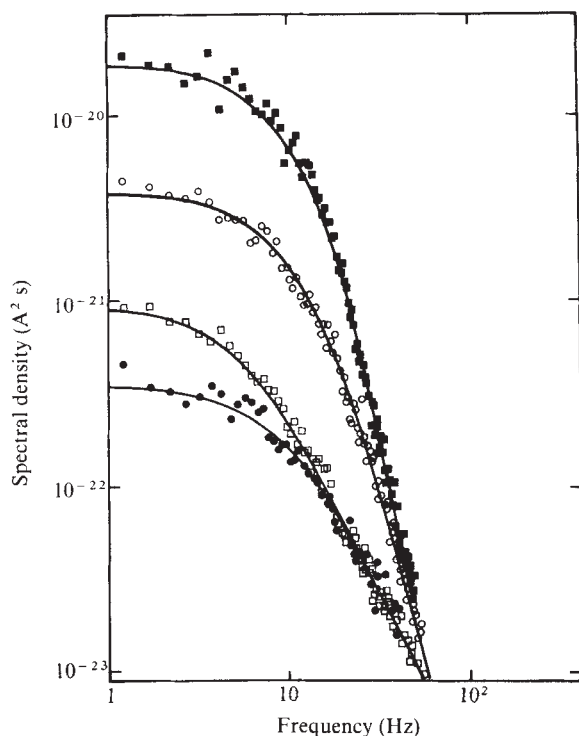


Fig. 2 The variance spectra calculated from the data obtained at 4 different light intensities. The variance spectrum corresponding to each stimulus intensity was calculated by a fast Fourier transform algorithm¹⁸ of 4,096 words based on 2.048-s data segments¹⁹. Data segments obtained at high light intensities were corrected for slow drifts before spectral estimation¹⁵. Normally, 50–100 data segments were used to obtain the variance spectrum. It can be seen that: (1) the amplitude of the spectral density decreases with increasing light intensity, (2) each of the spectra can be fitted quite well by an expression of the form $\sim 1/(1 + 4\pi^2 f^2 \tau^2)^m$, (3) the parameter m changes systematically from 3 to 1 as the light intensity increases, (4) the time scale of the bumps shortens with increasing light intensity, as indicated by the relative increase in the high-frequency components of the spectra with increasing light intensity. Interpretations of these data are discussed in the text. Values of $\log I/I_0$, m and τ (ms), respectively, are: ■, -6.5, 3, 8.44; ○, -5.5, 2, 11.5; □, -4.5, 1, 28.3; ●, -3.5, 1, 18.7.

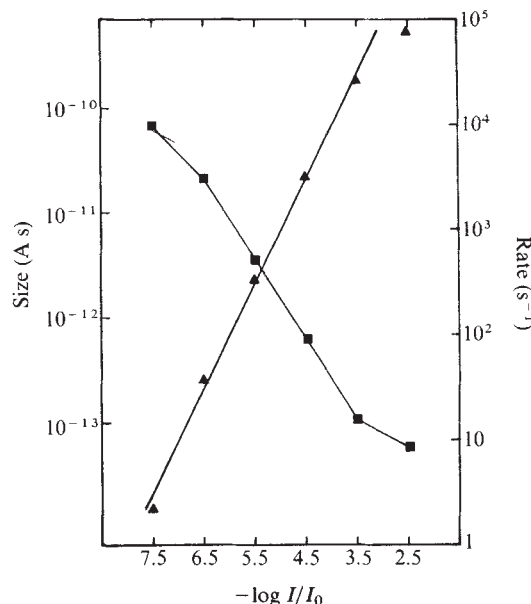


Fig. 3 The rate (λ) and the size of bumps at different light intensities calculated from the data. To calculate the mean, the 'baseline value' was routinely calculated from the baseline before and after the response. To calculate the variance, corrections were routinely made for contributions from the background (estimated from the baseline) and the slow drifts¹⁵. At $\log$ light intensity of -7.5, the calculated rate of occurrence is the same as that estimated from counting the number of bumps in each record. The size is the area under a bump so that it has units of A s. Over almost the full range of light intensities studied, the rate increases in strict proportionality to light intensity and then shows saturation at $\sim 10^5$ bumps s^{-1} . At $\log$ intensity of -7.5, the conductance increase due to a bump (the height) is $1.3 \times 10^{-8} S$, if the driving potential is taken to be 90 mV. Between -6.5 and -3.5 $\log$, the size decreases as the 0.77th power of light intensity. The conductance increase due to a single bump, at $\log$ intensity of -2.5, seems to approach a lower limiting value ($\sim 10^{-11} S$) (ref. 15). ■, Size $\sim I^{-0.77}$; ▲, $\lambda \sim I$.

Using these values we calculate that at the saturation intensity, $\sim 10^{-4}$ photons s^{-1} are absorbed by each rhodopsin. As these photons give rise to 10^5 bumps s^{-1} , there would have to be 10^9 'effective sites' in a cell at that intensity, as well as all intensities below it, because of linearity between intensity and bump rate. If each of these effective sites is a single channel, it is estimated that there are 10^9 channels in a cell, which corresponds to the estimated number of rhodopsin molecules in a single ventral photoreceptor²⁶ and is also consistent with the density of membrane particles observed at the microvilli of the ventral photoreceptors by freeze-fracture techniques (F. W. and H. B. Peng, unpublished results).

This research was supported in part by grants from NIH and NSF to William L. Pak. I thank Professors Bruce Knight and Pak for discussions.

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Lyt markers on thymus cell migrants

THE lymphocyte antigens Lyt-1 and Lyt-2 have been clearly shown to be differentially expressed on distinct, functionally defined subsets of precursor and effector T cells. The cell population expressing only Lyt-1 includes 'helper cells' and cells which react in mixed lymphocyte cultures. The population expressing only Lyt-2 includes 'suppressor' and cytotoxic cells, whereas cells bearing both antigens (Lyt-1,2) seem to function as precursors of cytotoxic and suppressor cells and in some systems, as amplifiers (reviewed in ref. 1). Peripheral T cells consist of about 50% Lyt-1,2 cells, 30-40% Lyt-1 cells and 10% Lyt-2 cells², although the proportions vary somewhat from organ to organ (R.S. and I.W., unpublished data). More than 90% of all thymic lymphocytes are immuno-incompetent and Lyt-1,2, but the relatively infrequent (less than 10% of all thymocytes) cortisone-resistant cells have properties of immuno-competence and Lyt phenotype distribution more like those of peripheral T cells (~45% Lyt-1,2, 50% Lyt-1 and <5% Lyt-2 (ref. 2, and R.S. and I.W., unpublished data)). Speculation concerning the phenotype of thymic emigrants has centred on the hypothesis that the thymus migrants are Lyt-1,2 precursor cells which differentiate peripherally, but no experiments directly testing this hypothesis have been published. Here we report experiments which demonstrate that cells leaving the thymus are already divided into subpopulations defined by the Lyt antigens.

With few exceptions³, published experiments concerning Lyt antigens have exploited the cytotoxic activity of the antisera as the assay for the presence of a particular antigen. However, as complement-mediated lysis is a secondary assay of antibody which is subject to variation due to antibody class and ineffective complementation, we have chosen to use immunofluorescence techniques. Similarly, we have developed a technique for fluorescein labelling of thymocytes *in situ*, to avoid problems associated with radioactive labels (such as long exposure times, stability of counterstains during photographic procedures, and differential uptake of label by cells of differing metabolic activity) and to facilitate automated analysis with the fluorescence-activated cell sorter (FACS). The technique is similar to that described previously using isotope labels⁴, and involves direct micro-injection of a solution of fluorescein isothiocyanate (FITC) in phosphate-buffered saline (PBS) into the thymus of anaesthetised mice. Thymus migrants can then be identified in cell suspensions or frozen sections of peripheral organs by the presence of membrane fluorescence. The phenotype of these rare but distinctive cells can be established by counterstaining with rhodaminated antisera.

Details of the technique and controls will be published elsewhere, but some controls should be mentioned here. (1) Following intrathymic FITC injection, fluorescein-positive cells in the periphery stain positively with anti-T reagents and negatively with anti-immunoglobulin (Ig) reagents (see Tables 1 and 2 and ref. 5). (2) Intraspinal injection of FITC results in the

Table 1 Lyt phenotypes observed in cell suspensions of pooled organs of mice

Cell type	Counter stain	Rhodamine			No. of F ⁺ cells counted	No. of F ⁺ cells per 10 ⁴ cells scanned
		% +	% ±	% -		
F ⁺ thymocytes	anti-Ig	1	1	98	100	(30% F ⁺ cells)
	anti-T	98	1	1	100	
	anti-1.2	84	8	8	100	
	anti-2.2	87	4	9	300	
F ⁺ blood cells	anti-Ig	0	0	100	50	7
	anti-T	100	0	0	50	4.5
	anti-1.2	96	3	1	100	4
	anti-2.2	21	1	78	100	5.9
F ⁺ lymph node cells	anti-Ig	2	0	98	50	2
	anti-T	100	0	0	50	2.5
	anti-1.2	100	0	0	100	3.6
	anti-2.2	30	0	70	100	3.8
F ⁺ spleen cells	anti-Ig	0	0	100	50	1.5
	anti-T	98	2	0	50	1.5
	anti-1.2	96	2	2	100	1.6
	anti-2.2	24	0	76	87	1.8

Results were obtained from 6 (BALB/c×B/J)F₁ mice, 3-4 weeks old, 3 h after intrathymic injection of FITC (1 mg ml⁻¹ in PBS, pH 7.4), 10 µl per lobe. Cell suspensions were divided into aliquots and stained on ice in PBS-containing azide, as follows. Anti-Ig; 1st stage normal mouse serum, 2nd stage rabbit anti-mouse Ig, 3rd stage rhodaminated sheep anti-rabbit (this group functions as normal serum control for anti-Lyt sera and as a B-cell stain). Anti-Lyt-1.2 and 2.2; as for anti-Ig, but specific antisera for 1st stage. Anti-T, 1st stage is a rabbit anti-mouse T-cell (see refs 7, 8), 2nd stage the same rhodaminated sheep anti-rabbit as in the other groups. All cells in suspensions were counted for rhodamine positivity (data omitted for simplicity), then scanned for fluorescein-positive cells and their rhodamine positivity checked. Rhodamine ± represents cells which could not be clearly categorised as positive or negative. The data represent one experiment. F⁺ indicates fluorescein-positive cells.

appearance of both anti-T- and anti-Ig-positive cells among the fluorescein-positive cells in blood and lymph nodes. (3) The number of fluorescein-positive cells in the periphery increases with time after intrathymic injection. (4) Thymectomy immediately after intrathymic injection prevents the appearance of fluorescein-positive cells in the periphery. (5) Injection of FITC intravenously or subcutaneously into normal mice, or into the thoracic cavity of thymectomised mice, does not give rise to significant numbers of fluorescein-positive cells in the periphery. (6) Light scatter analysis with the FACS (which for lymphocyte populations is related to cell size) of thymocyte suspensions after intrathymic injection can detect no significant difference between the fluorescein-positive and fluorescein-negative cells, indicating that labelling is quite random within the thymus. (The proportion of thymocytes labelled by this technique is usually ~30%, but ranges over 10-60%.) (7) Fluorescein-labelled thymocytes have the same distribution of Lyt antigens as unlabelled cells in the same suspensions. (8) The level of FITC substitution of thymus migrants is well below that which affects

Table 2 Phenotype distribution of migrant lymphocytes

Cell type	Counter stain	Rhodamine			No. of F ⁺ cells counted	Summary
		% +	% ±	% -		
All migrants (F ⁺ cells)	anti-Ig	1	0	99	600	Lyt-1 = 67%
	anti-T	99	1	0	600	Lyt-2 = 1-2%
	anti-1.2	97	1.5	1.5	500	Lyt-1,2 = 31%
	anti-2.2	31	2	67	787	

Pooled data obtained from three separate experiments identical to that described in Table 1. In addition, data from blood, spleen and lymph node were pooled to give total migrants, as no differences were observed between these cell sources.

in vitro viability or the cell-cell interactions involved in lymphocyte homing.

The anti-Lyt antisera used in the experiments were anti-Lyt-1.2 ((C3H×B6/Ly 1.1)F₁ anti-CE) and anti-Lyt-2.2 ((C3H×B6/Ly 2.1)F₁ anti B6)⁶. Both sera have been absorbed with thymocytes from appropriate congenic mice and their specifications have been extensively characterised with the FACS and fluorescence microscopy. Staining of thymocytes from congenic control mice and of syngeneic thymocytes with normal mouse serum as a first stage gave negative results.

The results of some of these experiments are shown in Tables 1 and 2. The time point 3 h after intrathymic injection represents the earliest convenient time for enumerating fluorescent thymic emigrants in the periphery. Approximately one-third of these have been outside the thymus for 1 h or less (and only a few as long as 3 h), and therefore, analysis of their Lyt phenotypes is probably a good reflection of the migrant population as it leaves the thymus. Table 1 shows the data from one such experiment. The proportions of the various Lyt phenotypes observed in the thymus correspond closely with those reported by others. Although the number of fluorescein-positive cells counted in the periphery is necessarily small, the results are clear-cut. About 75% of peripheral FITC-labelled cells are Lyt-1, and 25% are Lyt-1,2; Lyt-2 cells are extremely rare. There is no obvious difference between lymph nodes, spleen or blood; this was confirmed in two other experiments and the pooled data for all migrants seen are given in Table 2, with a summary of the phenotype distribution. The fact that this distribution is so different from that of the whole thymocyte population, taken together with the random labelling of thymocytes by FITC (see control 6 and 7 above), strongly suggests that this technique is actually revealing a physiological process and not a random spilling of thymocytes as a result of the experimental procedures.

These data do not provide any insight into the intrathymic pedigree of the migrants. Thus, it is not possible to determine whether the precursors of the Lyt-1 migrants are Lyt-1 thymocytes or whether they are Lyt-1,2 precursor cells. Lyt-2 cells of the cytotoxic and suppressor subclasses have been shown to be derived from peripheral Lyt-1,2 precursors (H. Cantor and R. Gershon, personal communication), and as Lyt-1,2 but not Lyt-2 cells are well represented among the thymus migrants, it is possible that the Lyt-1,2 migrants may only differentiate into Lyt-2 progeny. It is also likely that factors such as host age and recent antigenic experience may affect T cell subset representation among thymus migrants. For example, it is possible that stimuli which induce the production of Lyt-2 cells in the periphery may increase the proportion of this cell type among thymus migrants. Experiments to test these possibilities are in progress.

We thank Ms Libuse Jerabek for technical assistance. Anti-Ly antisera were prepared in conjunction with Dr D. Murphy in Dr H. McDevitt's laboratory. R.S. is a Special Fellow of the Leukemia Society of America; M.K. is supported by the Deutsche Forschungsgemeinschaft (DFG); E.B. is an NIH post-doctoral fellow (grant no. GM-02236); I.W. is a Faculty Research Awardee of the American Cancer Society (FRA 120). This work was supported in part by USPHS grant AI-09072 to I.W.

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Received 17 July; accepted 4 September 1978.

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Fibronectin-plasma membrane interactions in the adhesion and spreading of hamster fibroblasts

GLYCOPROTEINS of the cell surface have been implicated in intercellular adhesion and in the attachment of cells to a growth surface¹. However, the specificity of this role and the possibility of a direct involvement of carbohydrate are poorly understood. We have reported² that some lectin-resistant mutant cells^{3,4} defective in the biosynthesis of surface carbohydrates are poorly adhesive to serum- or gelatin-coated cover slips and show low cell-cell aggregation. A major glycoprotein component of serum, fibronectin^{5,6} (also called LETS⁷ and Cig⁸), analogous to a glycoprotein of adherent mammalian fibroblasts, has been identified as the active factor enhancing adhesiveness and spreading of cells to plastic and glass⁹⁻¹³ and collagen films¹⁴. We now report that fibronectins purified⁹ from culture fluids of normal BHK or ricin-resistant (Ric^R) cells mediate the rapid adhesion and spreading of wild-type BHK cells to plastic and to collagen films. By contrast, mutant Ric^R cells deficient in surface carbohydrates respond poorly to fibronectin in adhesion to these substrates.

Table 1 Total amount of fibronectin obtained from confluent cultures of hamster fibroblasts

Cell line	Amount of fibronectin (mg)
Wild type*	1.3
Ric ^R 14	1.2
Ric ^R 17	1.5
Ric ^R 19	1.2

Fibronectin was obtained from confluent cultures (620 cm² roller bottle) after 24 h collection in Waymouths medium¹⁸, precipitation with 70% saturated ammonium sulphate and passage through an anti-fibronectin column followed by chromatography on gelatin-Sepharose (ref. 9) and S.D.J.P. and R.C.H., manuscript in preparation). Protein concentration was estimated by A₂₈₀ and using the known extinction coefficient of fibronectin (A_{1%cm} 280 nm = 12.8)²⁵.

* Mean of three experiments.

BHK cells in confluent monolayers express moderate amounts of surface-associated fibronectin, which is removed by brief trypsinisation¹⁵. When trypsinised BHK cells were added to plastic dishes in the absence of serum, the cells retained a round shape for at least 3 h although time lapse cinematography showed intense membrane activity (Fig. 1a). After addition of fibronectin to coat the plastic⁹, the cells spread rapidly (Fig. 1a) and remained well spread indefinitely, becoming grossly extended as they moved over the substratum. Occasionally, an extension retracted into the cell body, presumably due to tension within the spread cells. However, no extended cell was seen to round up completely, such as occurs in mitosis. The cells did not multiply in Eagle's medium supplemented only with fibronectin over several days. A significant effect on cell spreading⁹ was obtained with fibronectin at 0.1 µg ml⁻¹ (Fig. 2a) and the cells were spread maximally after 45 min exposure to fibronectin at

approximately $3 \mu\text{g ml}^{-1}$. Spreading was not prevented by pre-incubation of cells for 1 h at 39°C with cycloheximide ($50 \mu\text{g ml}^{-1}$), actinomycin D ($5 \mu\text{g ml}^{-1}$) or chloramphenicol ($100 \mu\text{g ml}^{-1}$). However, cytochalasin B ($0.5 \mu\text{g ml}^{-1}$) inhibited cell spreading completely, and colchicine ($5 \mu\text{g ml}^{-1}$), although somewhat less inhibitory, clearly had an effect. We conclude, therefore, that normal BHK cells interact stably with a fibronectin film to form well extended shapes by a mechanism independent of new cell macromolecular synthesis but requiring a functional contractile system.

Ricin-resistant cell lines^{3,4} behaved in a significantly different fashion from wild-type BHK cells (Fig. 2a). First, the proportion of cells spreading in the absence of exogenous fibronectin was consistently lower ($<10\%$) than that found with wild-type cells. Second, the response to fibronectin at the lower concentrations was much smaller, particularly for Ric^R 17 cells. Time-lapse cinematography of the attachment of Ric^R 17 cells to fibronectin-coated dishes (Fig. 1b) showed that cells which seemed to be spread (and would be scored as such in Fig. 2a) frequently retracted to a round shape. Over several hours, individual cells were seen to spread reversibly many times. One possible interpretation is that the mechanism of interaction with fibronectin is defective in the mutant clones Ric^R 14 and Ric^R 17 and the cells cannot form the stable contacts necessary to maintain a well spread shape. Some support for this notion comes from surface labelling studies (S.D.J.P., G. Mills and R.C.H., submitted). Intact, untrypsinised Ric^R 17 cells lack surface-exposed fibronectin and Ric^R 14 cells express low levels compared to wild-type BHK or Ric^R 19 cells. However, these cells continue to synthesise and secrete fibronectin (Table 1), and fibronectins recovered from the culture medium induce spreading of wild-type cells (Fig. 2b). Therefore, the failure of Ric^R 14 and Ric^R 17 cells to retain surface-bound fibronectin is due to a defect in the cell surface and not to structural alterations in the fibronectin molecule. Ric^R 19 cells express normal amounts of surface fibronectin and respond more normally in the spreading assay (Fig. 2a).

The attachment of trypsinised BHK cells to collagen films was enhanced about fivefold by fibronectin, whereas Ric^R 14 and Ric^R 17 cells adhered to a significantly lesser extent (Fig. 3). At low concentrations of fibronectin ($<1 \mu\text{g ml}^{-1}$) Ric^R 19 cells attached significantly better than even wild-type BHK cells.

These results support previous evidence that Ric^R 14 and Ric^R 17 cells adhere relatively poorly to adsorbed serum proteins and to gelatin², and that fibronectin is the major component of serum mediating these effects⁹⁻¹⁴. Their relevance to the decreased cell-cell aggregation of Ric^R 14 and Ric^R 17 cells² is less clear, although fibronectin-cell interactions may be important here also^{16,17}. To summarise, cells with reduced levels of surface carbohydrate receptors for ricin^{3,4} synthesise and release fibronectin but cannot retain it at the cell surface. Such cells after trypsinisation respond poorly to exogenous fibronectin adsorbed to the bottom of a plastic culture dish or to collagen films. Ricin-resistant cells such as Ric^R 19, which express normal levels of ricin receptors, retain surface fibronectin and respond more normally in adhesions mediated by exogenous fibronectins. However, simple combination of fibronectin with surface carbohydrate receptors is insufficient to produce well spread, adherent cells. Spreading is strongly inhibited by cytochalasin B, suggesting that microfilaments are involved, perhaps in the recruitment of integral surface membrane receptors for fibronectin into a localised area of contact between the cell surface and the fibronectin film. Such adhesive plaques¹⁹ seem to be rich in actin filaments²⁰ and cell-coat material^{21,22}, and may be stabilised by cross-linking between fibronectin molecules by disulphide linkages⁶ and in some cases covalently by transglutaminase⁶.

It is not known whether fibronectin interacts with a single or several membrane glycoproteins. About 15 ricin-binding glycoproteins have been identified at the BHK cell surface²³. However, the specificity of fibronectin for carbohydrate receptors may be more precise than that of ricin, for several of the

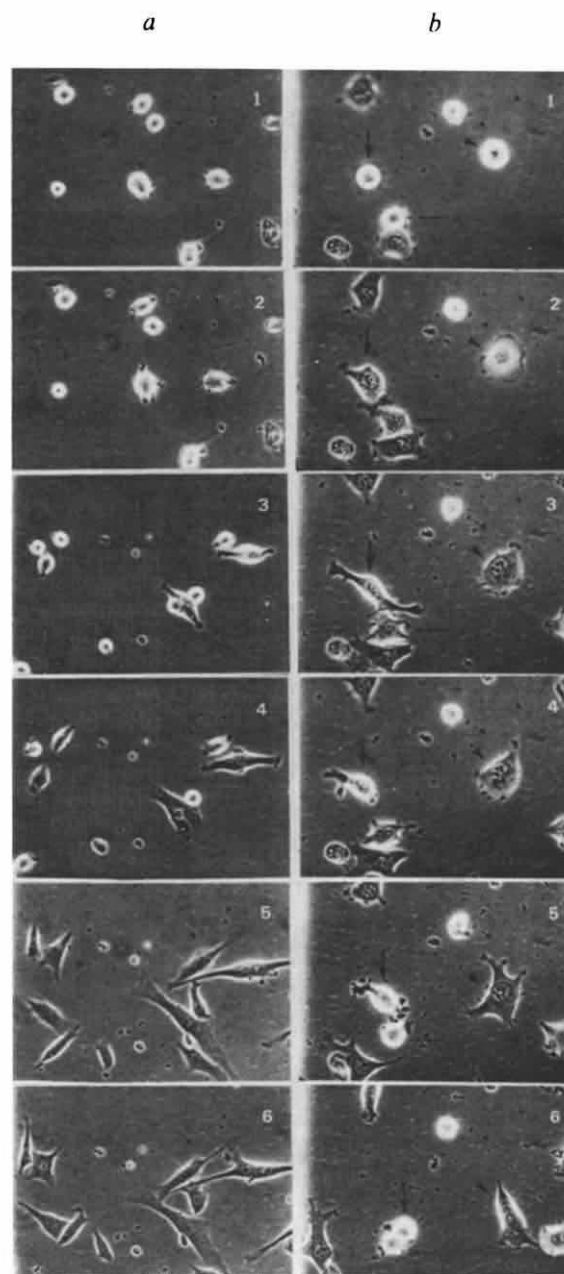


Fig. 1 Individual frames from time-lapse films showing the attachment and spreading on plastic of normal BHK cells (a) and ricin-resistant Ric^R 17 cells (b) mediated by hamster plasma fibronectin purified by gelatin-Sepharose affinity chromatography⁵. Cells were grown to confluence on plastic 75 cm² Falcon bottles in Eagle's medium plus 10% fetal calf serum, and dispersed with trypsin as described elsewhere^{4,9}. Trypsin was inactivated with medium plus 10% serum previously depleted of fibronectin by passage through a gelatin-Sepharose column^{5,9} and the cells were washed once with Eagle's medium minus serum and finally suspended in the same medium at 5×10^6 cells ml⁻¹. Cells (2×10^6) were added to 25-cm² Falcon bottles containing 5 ml Eagle's medium minus serum and fibronectin added ($3 \mu\text{g ml}^{-1}$) at 0 min. Pictures were taken (1 frame 10⁻¹ s) under phase, $\times 20$, at a, frames 1-6, respectively, 77 and 54 min before addition and 2, 25, 69 and 94 min after addition of fibronectin; b, frames 1-6, respectively, 10, 17, 29, 32, 60 and 93 min after addition of fibronectin. Note rounded cells with filopodia in wild-type cells before addition of fibronectin (a, frames 1 and 2). After addition of fibronectin, cells (different field) spread rapidly to a stable configuration (a, frames 3-6). Most Ric^R 17 cells spread to some extent but reverted to rounded morphology (b, arrows, same field throughout).

Graph (a): Shows the effect of fibronectin concentration on cell attachment for three cell lines: 3T3 (filled circles), 3T3-L1 (open squares), and 3T3-L1-2 (open triangles). The y-axis represents the percentage of attached cells (0-100), and the x-axis represents fibronectin concentration (0-10 $\mu\text{g ml}^{-1}$). All three cell lines show a dose-dependent increase in attachment with increasing fibronectin concentration. 3T3-L1-2 shows the highest attachment at higher concentrations, while 3T3-L1 shows the lowest.

Fibronectin ($\mu\text{g ml}^{-1}$)	3T3 (%)	3T3-L1 (%)	3T3-L1-2 (%)
0	~20	~10	~5
0.1	~35	~20	~18
0.3	~60	~35	~38
0.7	~75	~72	~48
3.0	~100	~92	~88
10.0	~95	~92	~95

Graph (b): Shows the effect of fibronectin concentration on cell attachment for three cell lines: 3T3 (filled circles), 3T3-L1 (filled squares), and 3T3-L1-2 (filled triangles). The y-axis represents the percentage of attached cells (0-100), and the x-axis represents fibronectin concentration (0-10 $\mu\text{g ml}^{-1}$). The x-axis has a break between 0.1 and 1.0 $\mu\text{g ml}^{-1}$. All three cell lines show a dose-dependent increase in attachment with increasing fibronectin concentration. 3T3-L1-2 shows the highest attachment at higher concentrations, while 3T3-L1 shows the lowest.

Fibronectin ($\mu\text{g ml}^{-1}$)	3T3 (%)	3T3-L1 (%)	3T3-L1-2 (%)
0	~18	~18	~18
0.1	~15	~22	~18
0.5	~40	~40	~32
0.7	~40	~55	~42
2.0	~72	~88	~75
10.0	~80	~90	~82

Fig. 2 Attachment and spreading of BHK cells and Ric^R cells on plastic dishes in response to fibronectin. Dispersed cells (see Fig. 1) were added to 35-mm dishes containing 2 ml Eagle's medium supplemented with hamster fibroblast fibronectins (Table 1). Dishes were preincubated at 39 °C for 1 h. After further incubation at 39 °C for 1.5 h the degree of spreading was estimated as the % of cells showing flattened nuclei and elongated processes. Each point shows the average of at least three determinations and indicates the range of values obtained for wild-type cells. Similar ranges were obtained for Ric^R cells. *a*, Spreading of BHK or Ric^R cells on normal BHK cell fibronectin. As small differences in biological activity were observed, only one batch of normal BHK-cell fibronectin (Table 1) was used in this experiment. ●, BHK; △, Ric^R 14; ○ Ric^R 17; □, Ric^R 19. *b*, Spreading of normal BHK cells on fibronectins from normal or mutant cells. ▲, Ric^R 14 fibronectin; ● Ric^R 17 fibronectin; ■, Ric^R 19 fibronectin; I, BHK fibronectin (range of values).

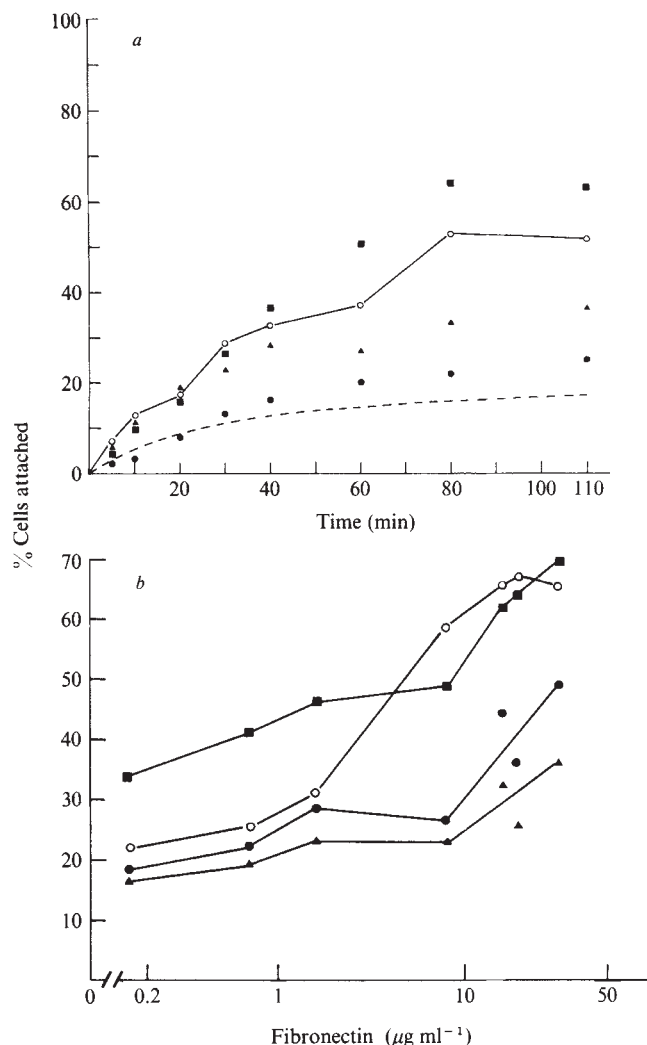


Fig. 3 Adhesion of normal BHK Cells and mutant Ric^R cells to collagen. Scintillation vials were treated¹⁴ with collagen (type III, calf skin, acid soluble). The cells were labelled by growth for 15 h at 39 °C in Eagle's medium plus 10% fetal calf serum and L-4,5-³H leucine (50 Ci mmol⁻¹, 10 µCi ml⁻¹) followed by a 5-h chase in nonradioactive medium. Trypsinised cells were washed into serum-free Eagle's medium (see Fig. 1) containing 10 mM HEPES buffer, pH 7.2. Cells (0.1 ml, 2 × 10⁵) were added to scintillation vials containing 1 ml Eagle's-HEPES medium minus serum and shaken reciprocally at 36 °C. Adherent cells were washed twice with phosphate-buffered saline and dispersed for radioactive counting²⁶. *a*, Collagenised scintillation vials were preincubated with hamster plasma fibronectin solution (19 µg ml⁻¹) in Eagle's-HEPES for 1 h at 36 °C. Control vials were preincubated in Eagle's-HEPES alone. ■, Ric^R 19; □, BHK cells plus fibronectin; ▲, Ric^R 14; ●, Ric^R 17; ---, BHK cells minus fibronectin. *b*, Collagenised scintillation vials were preincubated with various amounts of fibronectin for 1 h at 36 °C before addition of cells and then shaken at 36 °C for 1 h. □, BHK cells; ■, Ric^R 19; ●, Ric^R 17; ▲, Ric^R 14.

of adhesive plaques and perhaps part of the major surface-binding site for fibronectin.

Time-lapse photography was by John Clark, Clinical Research Centre.

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Intact cerebral ventricle as a site for tissue transplantation

TRANSPLANTATION of nervous tissue into the brain as a means of investigating both regeneration of the central nervous system (CNS) and graft viability has long been attempted^{1–3}. Typical methods of transplantation involve surgical removal of brain tissue in order to accommodate grafts or the direct insertion of grafts into the brain substance^{4–7}. Our approach differs from previous studies in avoiding mechanical damage to the brain surface or parenchyma. Thus, the regenerative capacity of the transplant and its interactions with intact brain surfaces, blood vessels and parenchyma can be studied without complication by appreciable scar formation or inflammatory response⁸. We report here the survival of allografted mature superior cervical ganglion (SCG) fragments into the intact IV ventricle of young rats. The transplanted autonomic fragments, initially without any afferent, efferent or vascular connections, regenerate vigorously as an *in vivo* culture within the cerebrospinal fluid (CSF). The undamaged IV ventricle provides a favourable site to study competitive interactions between brain and tissue grafts. We have found that the pattern of cell migration in developing cerebellum may be altered, so that cells of the external granular layer (EGL) and areas of maturing granular and molecular neuropil may migrate into the SCG graft. Entire laminae of EGL cells can be arrested at the cerebellar cortical surface even in 5-month-old rats. Normally, these cells complete their migration in the opposite direction, to their adult residence in the internal granule layer, before the end of the first post-natal month in the rat. This anomalous migration of developing CNS neurones through the limiting surface of the brain in response to a foreign tissue has not been previously reported.

SCG was removed from adult rats and immersed in biologically inert Pelikan ink in order to locate the implant after fixation. After decapsulating the ganglion and removing nerve trunks, a small fragment, about 1.0 mm³, was inserted through the cisterna magna with a polished glass rod. The sites of transplantation were chosen to ensure contact with either ependyma on the floor of the IV ventricle, the subpial glia or the cerebellar cortical surface (Fig. 1). Recipient rats were aged 6 to 14 days. One hour to six months after implantation the animals were perfused with aldehydes for light and electron microscopy. Serial 1-μm plastic sections were assessed for evidence of mechanical damage.

Within the first two weeks, the SCG fragments were well

vascularised by fenestrated vessels presumably from ganglion, choroid plexus or area postrema and by non-fenestrated vessels from meninges and brain parenchyma. In many instances the transplant was incorporated into the stroma of the choroid plexus and appeared continuous with normal brain parenchyma beneath the fourth ventricle (Fig. 2a).

Unexpectedly, in six animals where the implant lay close to the cerebellum, the migration of cells was profoundly affected. When confronted by SCG, many EGL cells either remain superficially or leave the cerebellum by a cellular bridge (Fig. 2c). Neither glia nor basal lamina form a barrier to the migration of cells out of the brain into the SCG graft.

Whole laminae of arrested EGL cells may be several cell layers deep and contain a heterogeneous population of granule cells, other neuronal types and glial cells (Fig. 2b). Note the invasion by granule layer neuropil, including apparently normal mossy fibres forming typical synaptic contacts and attachment plaques, into both the arrested laminae and the transplant itself. Islands of molecular layer neuropil also penetrate into the substance of the graft. In this zone of contiguity, only thin discontinuous glial slips may separate cerebellar tissue from SCG neurites and accompanying Schwann cells⁹. Where the transplant lies next to the medulla, the subpial glia facing the transplant participates in a subtle gliosis: astrocytic processes become arranged in many thin parallel sheets.

Within the transplant, bundles of axon sprouts separated by finger-like Schwann processes lacking basal lamina appear

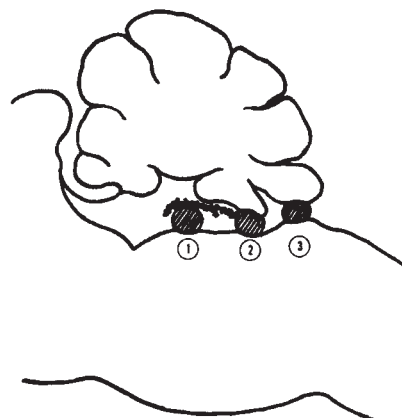


Fig. 1 Sites of transplantation. SCG graft (shaded circles) touches 1, floor of IV ventricle under choroid plexus 2, cerebellum over area postrema or 3, cerebellum over dorsal columns.

within a few days. Growth cones, (Fig. 2d) with comparable morphology to those in tissue cultured ganglia^{10,11} are recognised as late as six months, as are synaptic contacts between cell processes. Certain Schwann cells appear to be reactive in that their processes are replete with filaments and often interconnected by gap junctions¹².

Ganglion cells decrease in number with time, but many neurites become individually enclosed by several thin layers of Schwann processes. Between four and six months Schwann cells form typical compact peripheral myelin around a great many axons within the transplant (Fig. 2e). Some myelin sheaths appear to be in a developing stage. Schwann cells cooperate to form nodes of Ranvier. These normal discontinuities of the myelin sheath imply saltatory conduction within the transplant.

Originally, our attempt was to transplant newborn and fetal SCG, developmental stages mandatory for growth in tissue culture. These transplants quickly degenerated within the ventricle. However, the older the donor tissue, especially when older than three weeks, the greater the chance of survival and

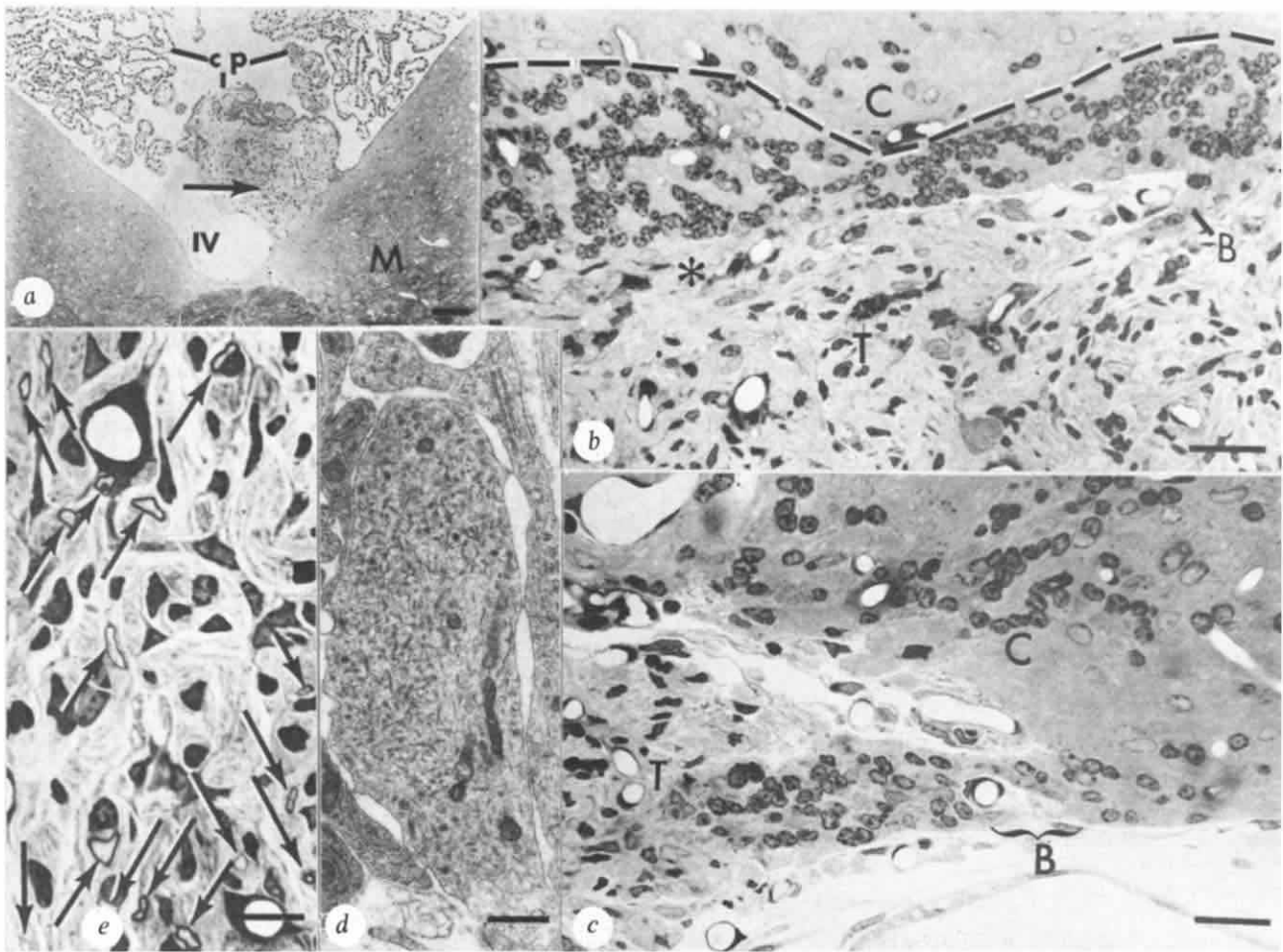


Fig. 2 *a*, Transplant (arrow) in the IV ventricle (IV) rests on the medulla (M) and is covered by choroid plexus epithelium (CP). 6 weeks. Scale bar, 50 μm . *b*, Transplant (T) is contiguous (asterisk) with cerebellum (C) which also forms a bridge (B) into T. Laminae of arrested cells of EGL (dashed line) face T. 4 months. Scale bar, 24 μm . *c*, Cerebellum (C) is connected with transplant (T) by a bridge (B) consisting of EGL cells and neuropil, 1 month. Scale bar, 22 μm . *d*, Growth cone in transplant. 1 week. Scale bar, 0.5 μm . *e*, Unlike normal SCG, many axons are myelinated in transplant; here 16 myelinated axons (arrows) are in a 8,000 μm^2 area of transplant. 6 months. Scale bar, 15 μm .

regeneration. This age requisite is quite different from that of tissue culture in that neonatal intraventricular explants died and older ones flourished. The adult SCG fragment underwent a complete reorganisation, regressing to a 'young' state composed almost entirely of neurites and growth cones before advancing to a mature state where almost every large axon is enclosed by Schwann processes.

Autonomic neurones in culture have specific requirements for nerve growth factor (NGF)^{13,14} or need the supportive properties of non-neuronal cells^{15,16}. The transplanted neonatal SCG should have the necessary complement of non-neuronal cells; yet within the CSF, these cells do not seem to support the growth of young SCG neurones as they do in culture. The failure of these young neurones to survive may also be due to their not having had the time to make contact with a proper target organ before transplantation¹⁷. If such contacts are made before transplantation, then the neurones can regenerate in a foreign environment. Certain retrograde factors from a target are needed for neurones to develop and regenerate¹⁸⁻²⁰. Regenerating neurites, once they reach a target site, could then trigger a series of metabolic events in autonomic neurones that allows them to re-develop and attempt to find another target. The formation of myelin in the transplanted SCG suggests that a message from not only the axons²¹, but from the targets they reach, could also stimulate some of the Schwann cells to produce myelin and nodes.

The unexpected result of this study is the reaction of the intact brain surfaces to the implantation of autonomic nervous tissue. At the external surface of the developing cerebellar cortex, neither the marginal glia nor their basal laminae prevent the cells of EGL and neuropil from freely entering the transplant. In certain mutant strains of mice, the absence of specific connections or deficiencies in the Bergmann glia cause heterotopia and eventual death of granule cells²² and after drug treatments or X-irradiation some ectopic granule cells persist²³⁻²⁵. In our preparations the proximity of autonomic transplants to the undamaged brain surface elicits three responses: an arrest of cell migration, migration in an anomalous direction, and proliferation of glial cell processes. Cerebellar neurones and non-neuronal cells remain in the upper molecular layer months after they would have normally migrated to deeper layers. This anomalous migration apparently takes place across an increased number of marginal glial processes. Whether this minimal gliosis and the stimulus for the arrest or anomalous migration may be triggered non-specifically by merely touching the brain surface with non-neuronal tissue such as muscle or salivary gland has yet to be determined.

This system in which the transplantation site is easily accessible with a minimum of trauma lends itself to the study of some underlying mechanisms of the regulation and growth of both central and autonomic neurones. The cultivation of neurones and their targets within this CSF compartment, which can be

readily irrigated by test substances introduced 'upstream' in the lateral ventricles, should provide further information on competitive interactions between foreign tissues and brain cells.

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Received 26 June; accepted 12 August 1978.

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Oxygens in DNA are main targets for ethylnitrosourea in normal and xeroderma pigmentosum fibroblasts and fetal rat brain cells

ONE of the major problems in the study of chemical carcinogenesis is to identify in cells or animals the nucleic acid adducts known from *in vitro* experiments. When normal or xeroderma pigmentosum (XP) human fibroblasts or fetal rat brain cells (FBC) in culture are treated with the carcinogen, ethylnitrosourea (EtNU), to an extent permitting good cell survival (2–5 ethyl groups per 100,000 bases), we find that the nature and proportion of the alkyl products in their DNA are remarkably similar to those found when DNA is alkylated with EtNU to a high extent *in vitro* (1 ethyl group per 100 bases). The products quantified in this study are ethylphosphotriesters, 7-ethylguanine (7-EtG), 3-ethyladenine (3-EtA), O^6 -ethyl-deoxyguanosine (O^6 -EtdGuo), O^2 -ethylthymidine (O^2 -EtThd), O^4 -ethylthymidine and O^2 -ethylcytosine (O^2 -EtC). We present here the first experimental evidence that pyrimidine oxygen atoms are major sites of reaction in cells treated with EtNU. A comparison of the present model experiments with purified DNA and poly(dG)·poly(dC) with earlier studies on single-stranded polynucleotides (refs 6, 10, Table 1) indicates that Watson–Crick base-pairing does not significantly hinder alkylation of the oxygens. This is in contrast to previous findings concerning the very low reactivity of base-paired nitrogens. We explain this on the basis that only the oxygens have a pair of electrons not directly involved in hydrogen bonding.

The chemical reactions of DNA with carcinogenic *N*-nitroso alkylating agents have been studied extensively *in vitro* but to a much more limited extent *in vivo* (see refs 1–4 for reviews). There are several principal reasons for this contrast. One is the technical problem of obtaining DNA with sufficient radioactivity from cells or tissues exposed to doses of alkylating agents that do not drastically change the physiological state of the

organism. Another is the lack, until recently, of information regarding the multiplicity of potential sites of modification in nucleic acids. Most other investigators have used neutral or acid hydrolysis to depurinate methylated or ethylated DNA and then analysed only for those alkyl products that were liberated, and stable, in such conditions. The derivatives included 1-alkyl A, 3-alkyl A, 7-alkyl A, 3-alkyl G, O^6 -alkyl and 7-alkyl G (ref. 1). When perchloric acid hydrolysis was used, 3-alkyl C was also detected¹. There was also evidence that alkyl phosphotriesters were formed^{5–8}. When EtNU is the alkylating agent, about 75–85% of the ethyl groups are bound to oxygen atoms^{5,6}. All the *O*-alkyl pyrimidines are labile, to varying extents, when heated at pH 1.5, 7.0 or 12.5 (ref. 9) and the alkyl group of phosphotriesters is labile when heated in strong acid at high temperature^{5,7}. Therefore, when acid hydrolysis is used to degrade alkylated DNA, many derivatives are destroyed to varying extents. In the present experiments the amount of each *O*-alkyl product in DNA, as well as the major sites of *N*-alkylation, the N–7 of G and the N–3 of A, were determined. Such analyses are possible only when the DNA is isolated in mild conditions, enzymatically digested at neutrality, and when all subsequent separations are carried out in conditions where the alkyl derivatives are stable⁶.

We have now treated three types of mammalian cells with [14 C]EtNU in cell culture in conditions of low toxicity: SV40-transformed human fibroblasts (GM 637), SV40-transformed XP fibroblasts (XP12RO) (group A) and fetal (18th day) BDIX rat brain cells. Low cytotoxicity was indicated by the fact that treatment of the cells did not significantly reduce cell survival or colony-forming ability. However, treatment with high concentrations of EtNU caused cell death, and this is termed high cytotoxicity. Concurrent experiments were also carried out using a high and cytotoxic level of EtNU in human fibroblasts, and *in vitro* ethylation of DNA from fibroblasts and the double-stranded polymer dG:dC.

The conditions of cell growth, ethylation, isolation of DNA and enzyme digestion are given in the legends to Figs 1 and 2, which show the chromatographic profiles of the enzyme digests of representative samples. A comparison of ethylation *in vivo* (Fig. 1) or *in vitro* (Fig. 2a), with varying extents of ethylation, shows that the several peaks containing the seven derivatives listed in Table 1 resembled each other in relative amounts. In each figure, the percentages shown represent the fraction of total radioactivity associated with peaks B, C and D, respectively. In two *in vitro* ethylation experiments, the fibroblast DNA with the higher level of modification (700 ethyl groups per 10^5 DNA-P) showed almost the same proportion of each alkyl product as did the sample with six ethyl groups per 10^5 DNA-P (Table 1). The fibroblasts which were given a cytotoxic amount of EtNU had 21 ethyl groups per 10^5 DNA-P, or about 6–10 times as much as with low toxicity, but in spite of this the pattern of ethylation was similar (Fig. 1d). Also, no significant difference was seen for the three types of cells treated with EtNU (Fig. 1a–c). In general, the distribution of ethyl products is, within the experimental error, similar both *in vivo* and *in vitro*, and both qualitatively and quantitatively different from that observed when other alkylating agents are used (reviewed by B.S.¹). We conclude that the affinity of EtNU for the various oxygen atoms in DNA is the same for intracellular DNA and extracellular purified DNA and that, at levels of ethylation which permit good cell survival, about 75% of the ethyl groups in DNA can be identified as being covalently bound to oxygen atoms. In all but one experiment, the order of products is ethylphosphotriesters > 7-EtG > O^6 -EtG, O^2 -EtT, 3-EtA > O^4 -EtT, O^2 -EtC, for both *in vivo* and *in vitro* treated samples.

Several points should be noted regarding these data. Earlier work on *in vitro* treatment of salmon sperm and HeLa DNA with EtNU was in conditions where over 1,000 ethyl groups per 10^5 DNA-P were introduced^{5,10}. However, the relative amounts of the *O*-alkyl derivatives, 7-EtG and 3-EtA were in general agreement with the present data (Table 1). The one exception is the higher value now found for O^2 -EtC. This is due to the fact

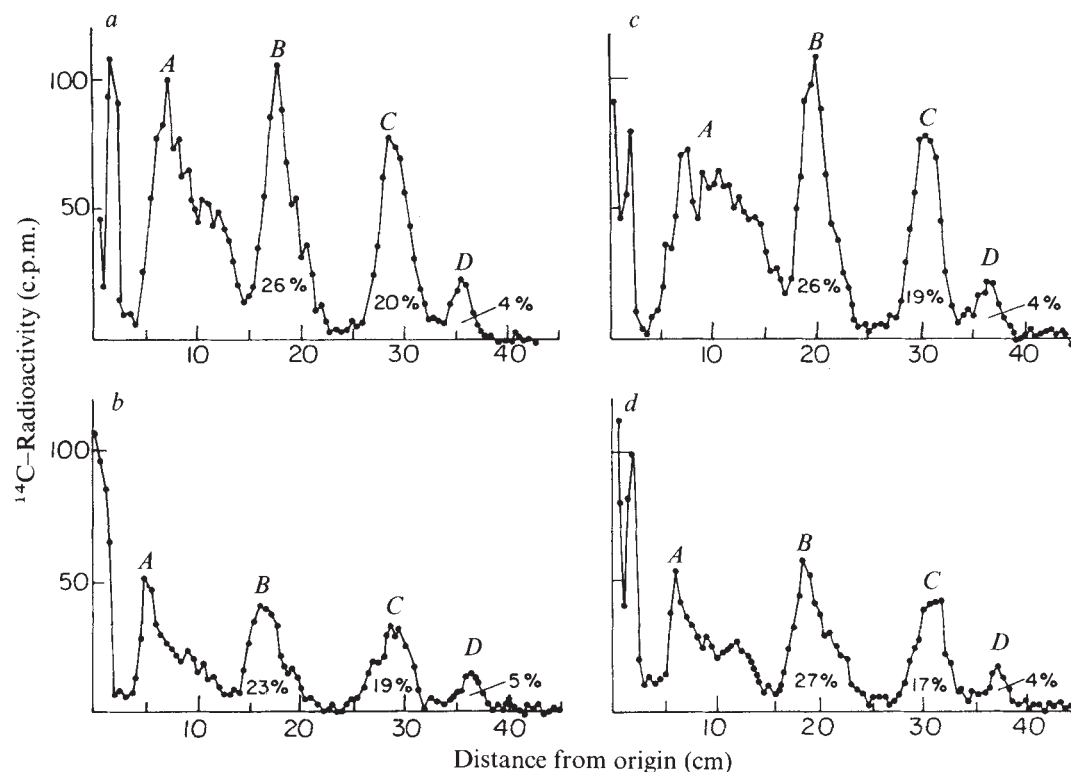


Fig. 1 Chromatographic profiles of enzyme digests of DNA from $[1-^{14}\text{C}]\text{EtNU}$ -treated cells. SV40-transformed human fibroblast cells (GM 637) (b,d; 3.4 and 21 ethyl groups per 10^5 DNA-P, respectively) and SV40-transformed XP cells (XP12RO) (c; 2.4 ethyl groups per 10^5 DNA-P) were grown in four roller bottles in Eagle's minimum essential medium plus 15% fetal calf serum, trypsinised, pooled, washed and resuspended in 10 ml phosphate-buffered saline (PBS) at pH 6.5. 2 mg $[1-^{14}\text{C}]\text{EtNU}$ (4.9 Ci mol^{-1} , Hoechst) in 0.1 ml dimethyl sulphoxide was added and the cells incubated at 37°C for 1 h. The cells were washed with PBS and frozen. Primary cultures of fetal BDIX-rat brain cells isolated at the 18th day of gestation (a; 5.6 ethyl

groups per 10^5 DNA-P) were grown in 75-cm² Falcon plastic flasks for 10 d, then treated with $150 \mu\text{g ml}^{-1}$ $[1-^{14}\text{C}]\text{EtNU}$ (5.2 Ci mol^{-1}) in PBS, pH 7.2, at 37°C for 30 min. The treated cells were washed with PBS, collected with 0.2% trypsin containing 0.02% EDTA and frozen in PBS containing 10% dimethyl sulphoxide. The number of cells obtained per experiment was approximately 3×10^8 cells. DNA was isolated and purified using a modified Marmur procedure¹⁸. The yield of DNA varied over 3–5 mg, with a specific radioactivity of 560–1,720 c.p.m. per mg DNA. In d the human fibroblast cells were treated with $1,500 \mu\text{g ml}^{-1}$ $[1-^{14}\text{C}]\text{EtNU}$ at 37°C for 1 h. The yield of DNA was similar to that in a, b and c, except that the specific activity was 6,400 c.p.m. per mg DNA. Enzyme digestion and chromatography of the various DNA samples were essentially as previously described⁶. DNA was dissolved in H_2O at a concentration of 10 mg ml^{-1} . Digestion was carried out as follows: per 100 μl of DNA, 20 μl of 0.5 M Tris, pH 7.3, 2 μl of 1 M MgCl_2 , 20 μl DNase I (5 mg ml^{-1} , 2,465 units per mg, Worthington), 20 μl snake venom phosphodiesterase (5 mg ml^{-1} , 32 units per mg Worthington), 8 μl bacterial alkaline phosphatase (5 mg ml^{-1} , 37 units per mg, Worthington) and 8 μl acid phosphatase (5 mg ml^{-1} , 16 units per mg, Worthington) were added. The samples were digested for 24 h at 37°C . At the end of digestion the sample was heated at 100°C for 5 min, and centrifuged to remove denatured enzyme protein. The entire sample, 0.3–0.6 ml, was applied in a streak 5–6 cm long on Whatman 3MM paper. Various ethyl nucleosides and bases were added as markers either to the enzyme digest before chromatography (Whatman 3MM with n-butanol-ethanol-water (80:10:25) as solvent, descending for 16–24 h) or as separate external standards. R_F values were as previously shown⁶. Peak A contains a portion of the alkyl phosphotriesters but quantitation of these products was more definitive using perchloric acid digestion and measuring the released alcohol⁵. Peak B coincided with 7-EtG and 3-EtA which were separated from each other, and from a small amount of unidentified material, by further chromatography in methanol:HCl:water (70:20:10). Peak C coincided with O^6 -EtdGuo and O^2 -EtThd which were separated from each other, and from any O^4 -EtThd (also found in peak D) by thin-layer chromatography⁶. Peak D was almost entirely O^2 -EtC but did contain a small amount of O^4 -EtThd. Thin-layer chromatography easily resolved these two derivatives⁶. After radioactivity measurement of the 0.5-cm paper strips, those strips in areas of chromatograms which were further studied (see above for resolution of peaks B, C and D) were washed free of scintillation fluid and eluted as previously described⁶.

The proportion of total radioactivity associated with peaks B, C and D is shown.

that a portion of O^2 -ethyldeoxycytidine (O^2 -EtdCyd) was converted to O^2 -EtC, thus necessitating that both derivatives be isolated. Our present technique of heating the enzyme digest at 100°C for 5 min converts all the O^2 -EtdCyd to O^2 -EtC (ref. 9), which on chromatography is easily separable from all other products (Figs 1, 2a; peak D). Furthermore, this same neutral heating procedure ensures that 7-EtG and 3-EtA are in the form of bases which together comprise most of the radioactivity in peak B (Figs 1, 2a).

In double-stranded DNA the O^2 of C, the O^4 of T and the O^6 of G are involved in hydrogen bonding to the complementary bases. It was an unexpected finding that there was considerable alkylation on the oxygens of the bases. Therefore, poly(dG)·poly(dC) was chosen as a model for examining the reactivity of the O^2 of C and the O^6 of G when firmly hydrogen-bonded to the complementary strand. However, poly(dG)·poly(dC) as the double strand is remarkably resistant to enzymatic digestion. We therefore developed a three-step method for the complete digestion of alkylated poly(dG)·poly(dC) in which O^2 -EtC is depyrimidinated at neutral pH; O^6 -EtG, 7-EtG and G are depurinated at pH 1.0,

and the remaining polymer is digested enzymatically as described in the legend to Fig 1. After removing any single-stranded regions by S_1 nuclease pretreatment, poly(dG)·poly(dC) was treated with EtNU in conditions where the double-helical structure is preserved, and it was found that 3% of the ethyl groups are bound to the O^2 of C and 16% to the O^6 of G (Fig 2b). Jensen (personal communication) has observed that the O^4 of T in poly(dA·dT) can be ethylated by EtNU, although to a much less extent than the O^2 of T. It is now accepted that even in native DNA molecules, thermal movement gives rise to some locally denatured regions¹¹, and this 'breathing' may account for the reactivity of normally base-paired sites. Although this mechanism probably accounts for the low reactivity of the N-1 of A or the N-3 of C in double-stranded polymers¹ it is unlikely to explain the fact that the reactivity of the base oxygen atoms is not appreciably different in single- and double-stranded polymers. The base oxygen atoms possess an electron pair which does not participate in the hydrogen bond and therefore could be available for modification. The difference in reactivity of a nitrogen and an oxygen, both hydrogen-bonded in a double-stranded polymer,

Table 1 Distribution of ethyl products after reaction of DNA in cells, or *in vitro* with [1-¹⁴C]EtNU

	Ethyl groups bound per 10 ⁵ DNA-P	Phospho- triesters	7-EtG	3-EtA	Ethyl derivatives				Total % identified
				% Of total ethylation*					
Intracellular alkylation of DNA									
Low cytotoxic									
Human fibroblasts†	1.8–3.4	51 ± 3	13.6 ± 1	4.5 ± 0.6	7.1 ± 1.5	9.2 ± 1.8	2.1 ± 0.8	4.5 ± 2	92
Xeroderma pigmentosum Fibroblasts‡	2.4–3.9	50	13	5	5.5	11	2.5	3.1	90
Fetal rat brain cells‡	2.9–5.6	49	12.3	4.7	7	7.6	4.3	3.4	88
High cytotoxic									
Human fibroblasts	21	51	13.6	5.2	5.5	9.8	3.5	2.9	94
In vitro alkylation of DNA									
Human fibroblasts§	6	60	6.5	4.1	4.5	5.7	6.1	4.5	92
Human fibroblasts	700	58	9	5.4	7.4	6.3	2.2	2.6	91
Salmon sperm	1,000	61	11	4.3	6	7	1.4	>0.5	92
M13 phage, single stranded¶					5	7	5	3	

* See Figs 1 and 2 for methods. 7-EtG and 3-EtA are from peak B, O²-EtT and O⁶-EtG from peak C, O⁴-EtT from peaks C and D, and O²-EtC from peak D. Ethylphosphotriesters are calculated from the amount of ¹⁴C-ethanol released with HClO₄ hydrolysis⁵ after subtracting the O-ethyl groups bound to bases. Not all known alkyl derivatives were quantitated in these experiments. Thus, the last column giving the % identified is the sum of the derivatives in the Table only.

† Average of four experiments.

‡ Average of two experiments, one of which is from a pool of two separate cell cultures treated with ¹⁴C-EtNU.

§ DNA isolated from human fibroblasts was treated as follows: 2 mg DNA in 1 ml 0.5 M cacodylate buffer, pH 6, was reacted with 1 mg ¹⁴C-EtNU for 45 min at 25 °C. All subsequent procedures were as described in Figs 1 and 2. Average of two analyses.

|| Data from ref. 10. Phosphotriester value corrected for O-alkylpyrimidines.

¶ Data from ref. 6.

poly(dG)·poly(dC), is shown in Fig 2b. In this experiment O²-EtC is found but N-3 EtC is not detected, whereas in a single-stranded homopolymer, poly C, also treated with EtNU, both N-3 EtC and O²-EtC are major products¹⁰.

The various sites of O-alkylation of bases in the DNA of cells treated in conditions of low cytotoxicity include the O⁶ of G, O² and O⁴ of T, and the O² of C (Table 1). O⁶-alkyl G was shown to mispair by Gerchman and Ludlum and, as suggested by Loveless¹², could be considered a mutational modification¹³. More recently, we¹⁴ have demonstrated that alkylation of the O²

or O⁴ of U (or T) is mutagenic as indicated by their mispairing when incorporated into polynucleotide templates. The alkyl phosphotriesters which represent half the bound alkyl groups have not been studied with regard to mutagenesis, although they do change the physical and biological properties of a polynucleotide^{15–17}.

With the exception of O⁶-alkyl G (refs 1–4), none of the O-alkyl products shown in Table 1 had been previously searched for in mammalian cells treated with carcinogens in conditions of low cytotoxicity. The new derivatives we now

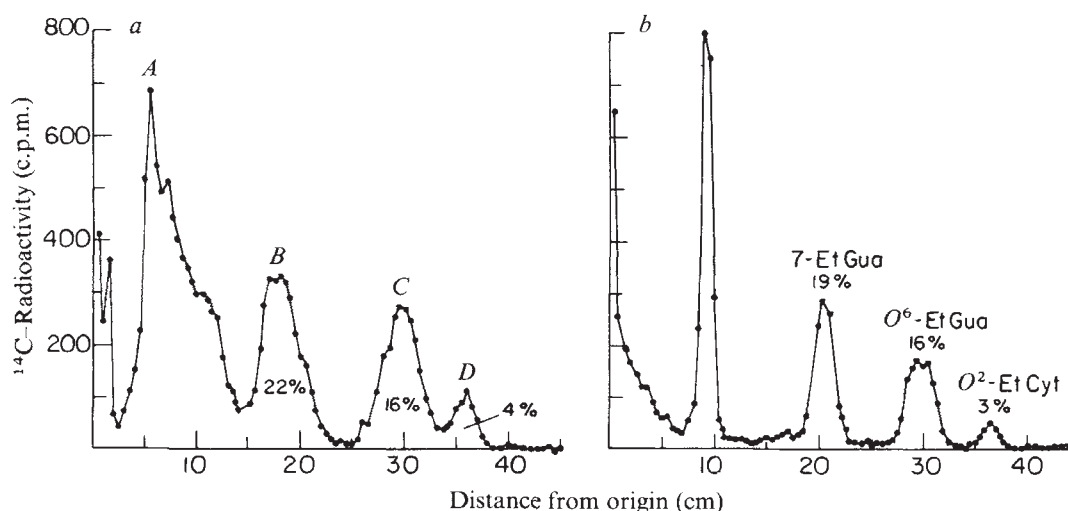


Fig. 2 Chromatographic profiles of digests of [1-¹⁴C]EtNU treated DNA from human fibroblasts (a, 700 ethyl groups per 10⁵DNA-P) and poly(dG)·poly(dC) (b, 160 ethyl groups per 10⁵PO₄). 2 mg of DNA from untreated human fibroblasts, isolated as described in Fig 1, was treated with 20 mg [1-¹⁴C]EtNU (1 Ci mol⁻¹) in 1 ml 0.5 M cacodylate buffer, pH 6.0, for 16 h at approximately 25 °C. DNA was freed of excess reagent by repeated alcohol precipitation, and digested with enzymes, followed by chromatography as described in Fig. 1. 10 A₂₆₀ units poly(dG)·poly(dC) (Miles) was treated with 1,000 units S₁ nuclease (Sigma) in 1 mM ZnCl₂, 0.1 M NaCl and 0.25 M sodium acetate at pH 5.0 for 90 min at 37 °C to remove any single-stranded polymer. The double-stranded polymer was extracted with chloroform–isoamyl alcohol (24:1), then precipitated with ethanol containing 0.4 M sodium acetate. The S₁-treated poly(dG)·poly(dC) was dissolved in 0.5 ml 0.5 M cacodylate buffer, pH 6.0, and reacted with 5 mg [1-¹⁴C]EtNU for 16 h at approximately 25 °C, then freed of excess reagent by repeated alcohol precipitation. Ethylated poly(dG)·poly(dC) was heated in water at 100 °C for 5 min to depyrimidinate O²-EtC (ref. 9). The pH was adjusted to 1.0 and the sample heated at 70 °C for 30 min to depurinate 7-EtG, O⁶-EtG and G. The remaining polymer was precipitated with ethanol containing 0.4 M sodium acetate and digested with enzymes as described for DNA. The ethanol supernatant from the two heatings was recombined with the enzyme digest before chromatography. In a peaks A–D are as described in Fig 1 and the subsequent separation into individual derivatives is the same as in Fig 1. In b the position of added unlabelled marker compounds and the % of total ethylation identified as 7-EtG, O⁶-EtG and O²-EtC are shown. The large peak about 7 cm from the origin in b represents ethylphosphotriesters but has not been resolved into those derived from dG and those from dC.

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report are found in DNA from cells which retain the ability to replicate and contain a total of two to five alkyl groups per 100,000 DNA-P. The similarity we find between *in vitro* and *in vivo* products with over a 1,000-fold difference in extent of reaction suggests the possibility of predicting *in vivo* reaction products of an ultimate carcinogen reacting with nucleic acids from the more easily performed *in vitro* studies.

The formation of *O*-alkyl bases is likely to cause mispairing during transcription or DNA replication and may be initiating events in cellular mutagenesis or neoplastic transformation. The fate of alkylation products in replicating cells is now under study.

This work was supported by grants from the NCI (B.S. and M.F.R.), US Department of Energy (J.E.C.), and Deutsche Forschungsgemeinschaft (M.F.R.). We thank I. Spratte and K. Shimizu for technical assistance, and Drs Manfred Kröger and H. Fraenkel-Conrat for suggestions and critical reading of the manuscript.

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Received 24 April; accepted 4 September 1978.

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Inhibition of protein synthesis by 2'-5' linked adenine oligonucleotides in intact cells

A SERIES of 2-5 linked oligoadenylic acid triphosphate (2-5A) inhibitors of cell-free protein synthesis are formed from ATP by an enzyme (2-5A synthetase) activated in interferon-treated cell extracts or rabbit reticulocyte lysates by double-stranded RNA¹⁻⁴. The major active species is the trimer, pppA2'p5'A2'p5'A. Cell-free protein synthesis is inhibited by subnanomolar concentrations of 2-5A and this inhibition seems to be mediated at least in part, by a nuclease which degrades mRNA⁵. However, 2-5A, the nuclease and the inhibition of protein synthesis are all unstable in cell-free systems. 2-5A is rapidly degraded in such systems prepared from control (or interferon-treated) cells, nuclease activity is transient and in the

absence of a 2-5A regenerating system protein synthesis resumes if fresh mRNA is added^{6,7}. Extracts derived from cells that have never been treated with interferon, therefore, have mechanisms for the synthesis of 2-5A (rabbit reticulocytes) and for responding to and degrading 2-5A. Thus, the 2-5A system, in addition to any role it has in the antiviral action of interferon, may also be involved in the regulation of normal cell growth and development. The extraordinary potency of 2-5A and its instability in cell-free systems makes the task of detecting 2-5A in intact cells very difficult. As an alternative we have studied the effect of exogenous 2-5A on protein synthesis in intact cells, using a recently described method for making animal cells reversibly permeable to small molecules⁸. Here we report that pppA2'p5'A2'p5'A and related 2'-5' linked oligonucleotides inhibit protein synthesis in hypertonically treated BHK-21 cells.

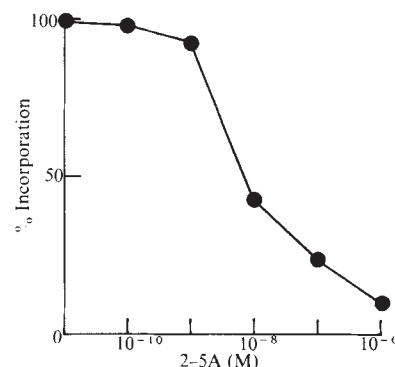


Fig. 1 Specific inhibitory activity of 2-5A on protein synthesis *in vivo*. BHK-21 cells (supplied by D. Pickup, National Institute for Medical Research) were grown in monolayer culture in Sterilin flasks and tissue culture dishes in Dulbecco's modified Eagle's medium containing 10% calf serum (Flow) at 37°C (in a humidified 5% CO₂ atmosphere). Cells plated at 2.5 to 5 × 10⁵ per 35 mm tissue culture dish and incubated for 20 h were permeabilised as described by Castellot *et al.*⁸ in medium minus serum containing 4.84% NaCl. When approximately 95% of the cells were permeable to staining by Trypan blue, the hypertonic medium was removed, the cells were washed with phosphate-buffered saline and 1 ml of complete medium minus methionine and containing 1 mM ATP 0.1 mM GTP, 100 mM creatine phosphate, ³⁵S-methionine (0.8 μCi ml⁻¹, 750-900 Ci mmol⁻¹) was added to the cells. Various concentrations of 2-5A (purified trimer, pppA2'p5'A2'p5'A) (ref. 4) were added and incubation was continued at 37°C. After 2 h the cells were washed twice with phosphate-buffered saline and 1 ml of cold 5% trichloroacetic acid was added for 30 min at 4°C. The cells were then washed three times with 5% trichloroacetic acid, digested in 1.0 ml of 0.1M NaOH for 30 min at 37°C and neutralised with 0.1 ml of 1.0 M HCl. The entire sample was counted by dilution into 10 ml of Tritonol¹⁰. Incorporation of ³⁵S-methionine into permeabilised cells in the presence of different concentrations of 2-5A is expressed as a percentage of the control value (no 2-5A). The total ³⁵S-methionine counts per min incorporated into the permeable cells in the absence of 2-5A was 159,645 c.p.m.

BHK-21 cells were made permeable by incubation in hypertonic medium as described by Castellot *et al.*⁸. When approximately 95% of the cells could be stained with Trypan blue, the hypertonic medium was replaced with complete medium containing ³⁵S-methionine and 2-5A at various concentrations. When incorporation of ³⁵S-methionine into acid-insoluble material was estimated after a 2 h incubation period it was found that an increasing concentration of 2-5A resulted in a decrease in incorporation of radioactivity (Fig. 1). In this experiment protein synthesis was inhibited by 50% in the presence of a concentration of 2-5A of less than 10 nM (in AMP equivalents). This is reasonable in comparison with the concentration required to give a 50% inhibition of cell-free protein synthesis (0.3-1 nM). The concentration required for 50% inhibition of

protein synthesis in the intact cells varied slightly between experiments (compare Fig. 1 with Table 1). Recently we found that this variation could be minimised by the addition of 2-5A to the cells during the hypertonic treatment.

The time course of incorporation of ^{35}S -methionine into permeabilised cells treated with 2-5A at 100 nM is shown in Fig. 2. Linear incorporation of ^{35}S -methionine into permeabilised cells was not observed until after 30 min of incubation in complete medium (Fig. 2) and was dependent on the presence of ATP and GTP (data not shown). Both the rate and extent of incorporation are inhibited when 2-5A is included in the incubation medium. Replacement of the hypertonic medium with warm complete medium allows resealing of the permeabilised cells to take place⁸. This resealing, as shown by a loss of Trypan blue staining, was not inhibited by 2-5A.

When 2-5A is synthesised with the 2-5A synthetase derived from interferon-treated cells and bound to poly(I). poly(C) Sepharose, an oligomeric series of products is obtained from dimer (pppA2'p5'A) to pentamer and occasionally hexamer and heptamer⁴. The predominant species is the trimer and this was used for the experiments described in Figs 1 and 2. The tetramer has a similar inhibitory activity to the trimer in the cell-free system. The dimer is almost inactive as is the bacterial alkaline phosphatase (BAP) resistant core of the trimer (A2'p5'A2'p5'A)⁴. When tested for activity using permeable cells, the trimer and tetramer were equally inhibitory (Table 1) but the dimer was very much less active, reflecting its lack of inhibitory activity in the cell-free system. There is, however, an interesting exception. Although it has no significant inhibitory activity in the cell-free system³, the nonphosphorylated core (A2'p5'A2'p5'A) seems to be only about 10-fold less inhibitory *in vivo* than the trimer or tetramer, suggesting that the cells can convert this core into active trimer by phosphorylation but that this function is lost in the cell-free system.

So far the 2-5A system has not been shown to have any preference in inhibiting virus-specific or host-specific protein synthesis in cell-free systems. Carrasco⁹ has shown that inhibitors of protein synthesis which do not penetrate uninfected cells

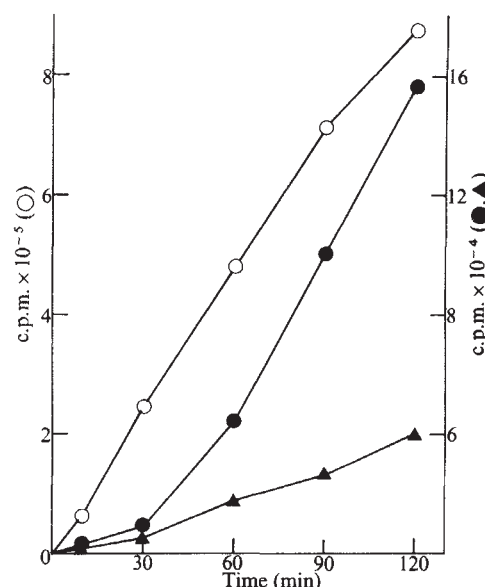


Fig. 2 Effect of 2-5A on the kinetics of protein synthesis in permeabilised BHK cells. Cell monolayers were made permeable as described in Fig. 1 and incubated in labelling medium in the presence or absence of 2-5A (purified trimer, pppA2'p5'A2'p5'A) at a final concentration of 100 nM. At the times indicated, control plates containing cells, not permeabilised (O), control plates containing permeabilised cells (●) and plates containing permeabilised cells incubated in the presence of 2-5A (▲), were removed, washed with phosphate-buffered saline and processed as described in Fig. 1. The rate and extent of incorporation of ^{35}S -methionine into control cells not treated with hypertonic medium was unaffected by the presence of 2-5A during the incubation period. (Data not shown.)

Table 1 Effect of different 2'-5' linked oligoadenylic acids on protein synthesis *in vivo*

Additions	Concentration (μM)	% inhibition of incorporation of ^{35}S -methionine
None	-	0
2-5A	50	83
(pppA2'p5'A2'p5'A)	10	63
	1	51
	0.10	39
Core (A2'p5'A2'p5'A)	50	64
	1	26
Dimer (pppA2'p5'A)	100	25
	10	0
Tetramer (pppA2'p5'A2'p5'A2'p5'A)	15	63
	1.5	44
	0.15	41

The 2'-5' linked oligonucleotides synthesised by 2-5A synthetase bound to poly(I). poly(C) Sepharose were partially purified on DEAE cellulose then fractionated into the oligomeric series by chromatography on DEAE Sephadex in the presence of 7 M urea⁴. The appropriate fraction corresponding to dimer, trimer and tetramer were pooled, dialysed, lyophilised and resuspended in water. The core (A2'p5'A2'p5'A) was prepared by complete chemical synthesis and was identical to core obtained by the action of BAP on the trimer (I.M.K., E.M. Martin and N. H. M. Birdsall, unpublished data). BHK cells were made permeable as described in Fig. 1, then incubated in labelling medium with the various oligomers of 2-5A at 37° for 2 h. The mean values from 3 experiments in which duplicate dishes were treated with the 2-5A oligomers are presented. The total ^{35}S -methionine counts per min incorporated into the permeable cells in the absence of any oligomer was 440,109 c.p.m.

are able to enter infected cells and to inhibit viral protein synthesis specifically because of changes in the permeability of the infected cell membrane. Despite the attractiveness of this approach we have not observed any inhibition of viral protein synthesis with dimer, trimer or BAP core in BHK-21 cells or mouse L-cells infected with either Semliki Forest virus or encephalomyocarditis virus under the conditions described by Carrasco (B.R.G.W. and G. Stark, unpublished observations). Presumably, this result is obtained because these oligomers exceed the molecular weight limit of about 750 for penetration into infected cells⁹.

The results presented here clearly demonstrate that 2-5A is a potent inhibitor of protein synthesis in the intact cell, a phenomenon previously observed only in cell-free systems. Moreover, the demonstration of the effectiveness of the non-phosphorylated core (A2'p5'A2'p5'A) in the intact cell in contrast to its inactivity in cell-free systems, emphasises the potential value of the permeabilised cell in screening related compounds for activity. Its major value, however, may be in determining whether 2-5A acts in the intact cell through a nuclease which degrades mRNA⁵ or has alternative or additional mechanisms of action.

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Received 7 August; accepted 18 September 1978.

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Plasmid-determined tetracycline resistance involves new transport systems for tetracycline

TETRACYCLINES are broad-spectrum bacteriostatic antibiotics which act by inhibiting protein synthesis^{1,2}. However, their usefulness in combating bacterial infection has been sharply curtailed by the widespread occurrence in bacteria of tetracycline resistance encoded by genes located on extrachromosomal DNA elements called plasmids^{3,4}. In many bacteria, notably *Enterobacteriaceae*, *Pseudomonas* and *Staphylococcus*, plasmid-mediated tetracycline resistance is inducible; the resistance level can be increased by preincubation of the cells in sub-inhibitory amounts of tetracycline^{5–8}. Coincident with induced resistance is the induced synthesis of a plasmid-encoded inner membrane protein which we have designated TET protein^{8–10}. Synthesis of this protein (and presumably most of the resistance determinant) is negatively regulated; a repressor has been partially purified¹¹. However, the mechanisms for plasmid-mediated tetracycline resistance are not yet clear, and there seems to be no degradation of the antibiotic in resistant cells^{10,12,13}. Although resistant cells accumulate less tetracycline than do sensitive cells^{14,15}, the moderately reduced uptake does not explain the much larger difference in sensitivity to the drug^{10,16,17}. We report here that plasmid-containing resistant cells take up tetracycline by a different transport mechanism from that of sensitive cells. This altered transport seems to be responsible for at least part of the difference in tetracycline inhibition of sensitive as compared to resistant cells.

Both sensitive and resistant isogenic *Escherichia coli* K12 strains displayed a biphasic uptake of ³H-tetracycline in enriched medium (Fig. 1a)¹⁸. In comparisons of sensitive and resistant cells, the initial 'rapid' uptake rate of the sensitive cells was reduced 3–10-fold by the induced tetracycline resistance plasmid R222 (which also carries resistance to chloramphenicol, streptomycin and sulphonamides) (Fig. 1b), and the second, 'slow' uptake rate was reduced 3–5-fold. Similar results were seen for R222 in other strains of *E. coli*, for the different tetracycline resistance plasmids R64 and pSC101, and for cells harbouring the tetracycline-resistance determinant transposed to the bacterial chromosome (DU3083, ref. 19). Conversely, in D1–8 cells, which harbour a mutant R222 plasmid sensitive to tetracycline, but bear all other resistance determinants, uptake was similar to that in the isogenic plasmid-less cells. Tetracycline was not irreversibly bound to either cell type, but was lost rapidly from the cells on dilution into tetracycline-free medium (Fig. 1). This finding is consistent with the rapid recovery on dilution into drug-free medium of cells treated with the drug.

As induction leads to markedly increased tetracycline resistance, we compared the uptake of tetracycline in induced and uninduced resistant cells. In uninduced cells the rapid uptake was intermediate between that of sensitive and induced resistant cells; the slow uptake resembled that of induced cells. Hence, the primary effect of induction was to reduce the rapid uptake.

Whereas 2,4-dinitrophenol (DNP), an inhibitor of oxidative phosphorylation, did not affect the rapid-uptake system in sensitive cells, it enhanced that in induced resistant cells (Fig. 2). Anaerobic conditions (bubbling the culture with nitrogen) also enhanced the rapid uptake only in resistant cells (data not

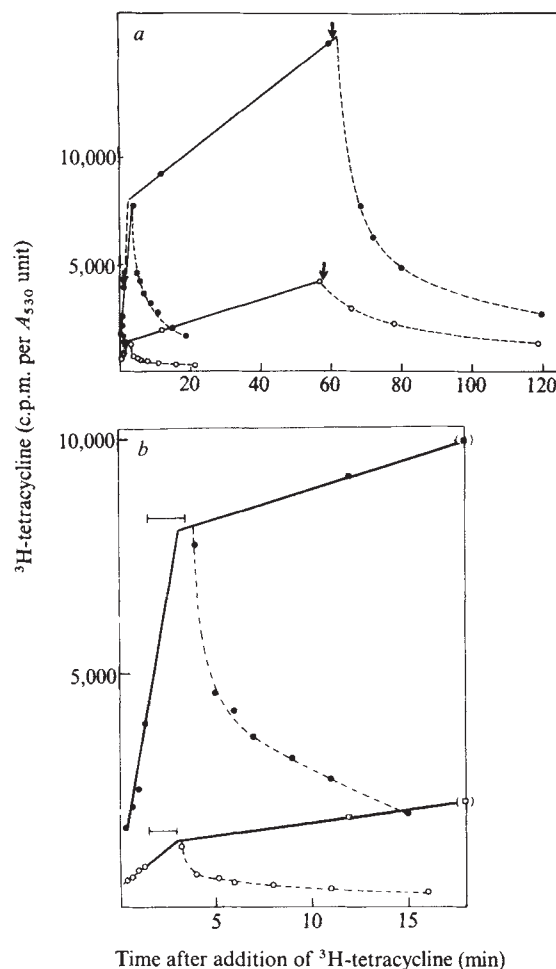


Fig. 1 Uptake and loss of tetracycline from sensitive and resistant cells. *a*, Uptake over 1 h. *E. coli* K12 strain D0-1 (identical with CSH-2 of T. Watanabe) and its plasmid R222-containing derivative D1-1 (induced to high-level tetracycline resistance by growing with 0.5 $\mu\text{g ml}^{-1}$ tetracycline for at least 30 min) grown at 37 °C in L broth²⁴ to mid-to-late log phase ($A_{530} = 0.5\text{--}1.0$ using a Coleman 101 spectrophotometer) were collected by centrifugation and resuspended in fresh L broth at 37 °C to an A_{530} of 4.5 (2×10^9 cells per ml). At various times after addition of 1.2 $\mu\text{g ml}^{-1}$ ³H-tetracycline (New England Nuclear, 0.6–1.0 Ci mmol⁻¹; freshly dissolved in methanol and added to approximately 10^6 c.p.m. ml⁻¹), samples were removed and diluted 1/17–1/25 into ice-cold L broth. The cells were then collected by centrifugation and their radioactivity determined¹⁸. Uptakes are presented as c.p.m. per A_{530} unit of cells. Where a concentration C of radioactive tetracycline other than 1.0×10^6 c.p.m. ml⁻¹ (at a 35% counting efficiency) was added to the cells, the uptakes were multiplied by 10^6 per C to normalise. At 1.5 and 58 min (arrows), part of the culture was filtered, resuspended to the same A_{530} in L broth containing no tetracycline, and loss of label from the cells was observed. *b*, Initial tetracycline accumulation. Data from *a* were plotted on an extended time scale. The horizontal bars represent the time taken to filter and resuspend those samples in which efflux was monitored. ●, ■, Tetracycline-sensitive cells; ○, □, tetracycline-resistant cells.

shown). Arsenate, an inhibitor of ATP synthesis, showed no such effect. These results suggest that resistant cells may have an energy-dependent (but not ATP-dependent) mechanism for blocking entry or stimulating efflux of the drug.

The slow-uptake system in induced resistant cells was not inhibited by DNP, sodium cyanide (CN), an inhibitor of respiration, or by arsenate (Fig. 2), whereas in sensitive cells, all these inhibitors stopped accumulation of tetracycline by the slow system¹⁸. The previous suggestion that arsenate inhibited the

slow-uptake system in tetracycline-resistant cells¹⁰ has not been confirmed and we conclude that arsenate causes little if any change in either the rapid- or slow-uptake system.

There are two major energy sources for transport of amino acids and some sugars in *E. coli*: the proton motive force and ATP (or a derivative) from oxidative phosphorylation or glycolysis^{21,22}. ATP-dependent uptake involves shockable (releasable) periplasmic binding proteins²¹. We found that the slow uptake of the sensitive cells was eliminated by osmotic shock, whereas that of the resistant cells remained intact (Fig. 3). Shock treatment did not appreciably change sensitivity of protein synthesis (as assayed by incorporation of ³⁵S-methionine into acid-precipitable material)²³ to tetracycline by either sensitive or resistant cells.

Using tetracycline concentrations up to 0.4 mM (200 µg ml⁻¹), no saturation was found for the rapid-uptake system of either sensitive¹⁸ or resistant cells. At this external tetracycline concentration, there did not seem to be saturation of the slow-uptake system in sensitive cells, but that in the resistant cells was found to be saturable with a *K_m* between 0.15 and 0.5 mM tetracycline and a maximum velocity of 6–16 ng per min per *A*₅₃₀ unit of cells (300 µg protein) as estimated from several experiments by nonlinear regression fit of data to the Michaelis–Menten equation.

These findings demonstrate that the plasmid-borne tetracycline-resistance determinant brings about dramatic changes in the inherent tetracycline uptake systems of sensitive *E. coli*: (1) reduced uptake and accumulation by the rapid-uptake system which is further decreased by induction to high level resistance; (2) replacement of the energy-dependent, shockable, slow-uptake system with an energy-independent, unshockable, saturable uptake system having a lower velocity at a given tetracycline concentration, as well as a lower *K_m* and maximum velocity.

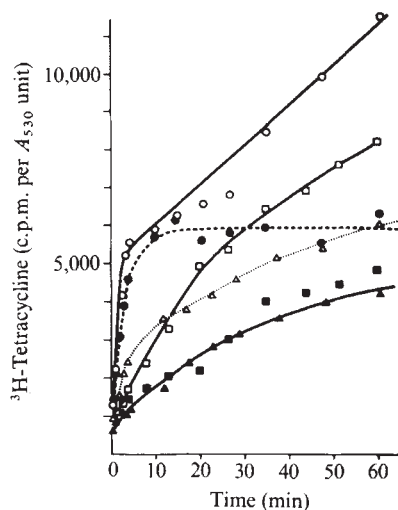


Fig. 2 Effect of inhibitors on uptake. D0-1 cells (*Tet^S*) and induced D1-1 cells (*Tet^R*) at an *A*₅₃₀ of 4.5 were preincubated for 17 min with or without 0.7 mM 2,4-dinitrophenol (DNP) before ³H-tetracycline addition at 1 µg ml⁻¹. In a separate experiment, D1-1 cells were preincubated for 10 min with or without 2 mM sodium cyanide (CN) before the addition of tetracycline at 0.9 µg ml⁻¹. Uptake was measured as described in Fig. 1, except that samples were diluted into cold buffered saline instead of L broth. In the cyanide experiment, cells were not centrifuged but were filtered through polycarbonate 0.4-µm filters (Nucleopore)¹⁸. Zero time (background) counts were determined on cells labelled for 30 s at 0°C. The data were corrected for different counting efficiencies to a uniform efficiency of 20%. ○, *Tet^S* control; ●, *Tet^S*+DNP; ▲, *Tet^R* control; △, *Tet^R*+DNP; the separate experiment ■, *Tet^R* control; □, *Tet^R*+CN.

In sensitive cells, the apparent intracellular concentration of drug during rapid uptake is best explained by diffusion accompanied by intracellular 'binding' of tetracycline which reaches an equilibrium with unbound drug¹⁸. Resistant cells may oppose this rapid uptake of tetracycline by reducing entry, further limiting the binding and/or introducing an efflux system. In the present test conditions, however, the efflux rate of resistant cells did not seem to be greater than that of sensitive cells (Fig. 1 and unpublished data). The slow-uptake system for tetracycline in sensitive cells is an active process¹⁸, whereas that in resistant cells has the properties of facilitated (carrier-mediated) diffusion. Tetracycline taken up by the latter system is concentrated minimally, if at all, which is consistent with a diffusion

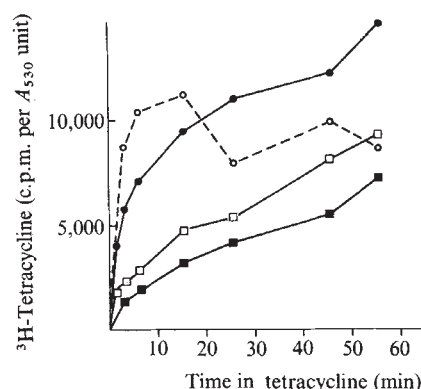


Fig. 3 Effect of osmotic shock on uptake. Late log phase D0-1 (*Tet^S*) and induced D1-1 (*Tet^R*) cells were collected, washed and resuspended at 23°C in 0.03 M Tris-HCl (control) or 0.03 M Tris-HCl with 20% sucrose (shocked) at pH 7.3. To the latter, 0.1 mM EDTA was added. Both sets of cells were incubated for 6 min at 23°C, collected and resuspended in cold 0.03 M Tris-HCl, pH 7.3 (control) or cold 0.5 mM MgCl₂ (shocked) according to the methods of Heppel²⁶. Cells were collected, resuspended in L broth at 37°C and ³H-tetracycline (0.6 µg ml⁻¹) was added. Uptake was measured on samples diluted and centrifuged in cold buffered saline. ●, *Tet^S* control; ○, *Tet^S* shocked; ■, *Tet^R* control; □, *Tet^R* shocked.

mechanism. In 30 min, at an external tetracycline concentration of 0.5 µg ml⁻¹, about 0.002 µg tetracycline per *A*₅₃₀ unit of cells is taken up, corresponding to 1.7 µg ml⁻¹ tetracycline within the cell, assuming 1.2 µl per *A*₅₃₀ unit. Although the ratio of 1.7:0.5 indicates that the drug is concentrated threefold within the cell, the numbers used are only estimations, and it is possible that tetracycline is, in fact, not concentrated by the cells.

It is not entirely clear how these plasmid-mediated changes contribute to tetracycline resistance. By a mechanism unusual for antibiotic resistance, the tetracycline-resistance determinant codes for the appearance of a new uptake system for tetracycline (as well as a new possible export system). As tetracycline probably enters the sensitive cell through a transport system meant for another cell substrate, the plasmid may replace this system by one with a similar affinity for that substrate, but less affinity for tetracycline. Furthermore, although accumulation is only reduced severalfold, the new transport systems could contribute more than a severalfold increased resistance if they placed tetracycline into less inhibitory sites within the cell. As the TET protein is located in the inner membrane, it is likely to be involved in the new transport systems.

If localisation of tetracycline in less inhibitory intracellular regions does not play a part in determining tetracycline resistance, other mechanisms would be needed to explain the 100–200-fold decreased sensitivity of induced resistant

cells in the face of a less than 10-fold difference in uptake. Recent *in vitro* protein synthesis data suggest that intracellular insensitivity to tetracycline activity may also be involved in the resistance mechanism^{10,27}.

This work was supported by grant VC 202 from the American Cancer Society. DU3083 was obtained from T. Foster, and CSH-2 cells from T. Watanabe.

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Received 26 June; accepted 29 August 1978.

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Homology between human and simian repeated DNA

A RELATIVELY small proportion of the total DNA of eukaryotic cells has been considered to be transcribed and translated; the rest of the genome may be variously categorised into several subsets such as spacer and repeated DNAs. Such nontranslated sequences presumably have a role in the modulation of transcription and in the organisation of chromosomes. Some satellite DNAs may be extremely simple in sequence¹, even in human DNA². Other repeated DNAs showing greater sequence complexity have been considered to form a separate subset of repeated DNAs^{2,3}. Very recently, the exact base sequence of such complex repeats has been determined in a few organisms^{4,5}. The conservation of such sequences between related species, and even more extensively in the evolutionary tree, is not known. We report here data which indicate the base order of a subset of human repeated DNA which is fairly complex in sequence. We have shown that such human DNA repeats are quite well conserved both in nucleotide sequence order and in nominal repeat length between human and lower primates.

The identification, isolation and purification of fragments of these human complex repeated sequences have been

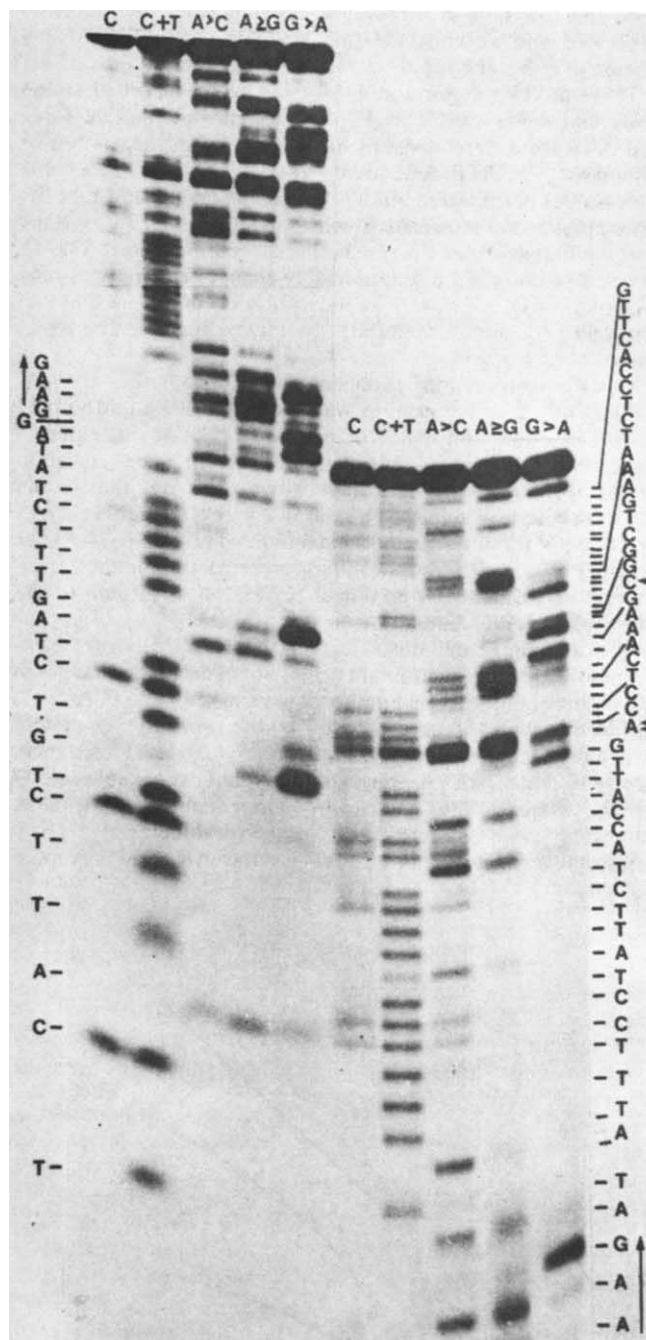


Fig. 1 Maxam-Gilbert¹⁷ procedure for chemical cleavage for DNA sequence of the fragment from *Hin*FI site (labelled with ³²P) to *Eco*RI* site (see Fig. 3). The 342-base pair fragment eluted from gel was first digested with *Mbo*I yielding 52- and 290-base pair fragments; the eluted 290-base pair fragment was subsequently cleaved with *Hin*FI to give a 121-base pair fragment. The eluted 121-base fragment was labelled at both 5' ends with ³²P using polynucleotide kinase as described² and then cut with *Eco*RI* to give the 80-base pair fragment shown (complementary to bases 97-175, Fig. 3). Electrophoresis was carried out at 1,000 V on a 1.2-mm thick, 12.5% acrylamide-urea gel, with overall dimensions 35 cm × 70 cm. Lanes on the right depict cleavage products electrophoresed for 19 h to resolve the higher molecular weight fragments. Lanes on the left show the same cleavage products electrophoresed for 9 h on the same gel. On this gel the sequence begins on the left at the complement to base 172 and proceeds to base complement 99, the last band that can be resolved with certainty. Upward arrows indicate overlap as one reads up the gel. The single arrow on the upper right indicates the presence of a 5-methylcytosine residue in the sequence (note gap, complementary strand position showed a G). The double arrow indicates a rare base variant, possibly a N⁶-methyladenine; complementary strand showed a T in this position.

described^{2,3} and more extensive data on sequence variations within these multiple fragments will be presented elsewhere. A 342-base pair fragment was obtained approximately 75% pure by eluting a band from gels after *EcoRI* digestion of total placental DNA. This band constituted roughly 0.75% of the total DNA of the genome and was further purified by cutting the eluted fragment with other restriction enzymes. The sequence of this entire molecule was determined using the Maxam-Gilbert procedure (Fig. 1). Other methods such as two-dimensional chromatography (Fig. 2) were used to corroborate these results. One strand of the double helix reading through *MboI* and *EcoRI** restriction sites to the centre of the 342-base pair molecule at the *HinfI* site, where the tandem repeat begins, is given in Fig. 3. For comparison, the comparable strand reported from the African green monkey repeated sequence⁵ is given just below this.

The 342-base pair dimer was found to consist of two tandem repeats of 171 nucleotides each. As more than one molecule was being sequenced and the sequences are resolved on gels by restriction sites, some variability at each base position could be expected. With the exception of five of the bases indicated in Fig. 3, the sequence data revealed essentially a single base for each position, as single spots were obtained on two-dimensional chromatography (Fig. 2) and unambiguous lines were seen on base-specific cleavage gels (Fig. 1).

In comparing the human sequence to the monkey sequence, two striking observations can be made: (1) the length of the repeat is virtually identical in the human and monkey sequence and differs only by one base or 0.6% of the total repeat length, and (2) 65% of the base sequence order is the same in monkey as in the human sequence, with only a single base insertion in the monkey sequence, most probably corresponding to position 126 of the human sequence (Fig. 3). Furthermore, it can be seen (Fig. 3) that there are clusters of base changes, especially in the centre of the molecule, and such changes can be used to test various models of repeated sequence evolution (in preparation). There is a 3% greater total GC content in the monkey than in the human sequence, thus explaining the relative position of the monkey repeat on density gradients as a satellite⁶.

Interestingly, the human sequence, like that of the monkey, contains many stop codons in all frameshifts and reading in both directions (seven to eight total in each direction). There is only one usual initiation codon (ATG), although unusual initiators (GTG) are seen in five places on the sequence (three to five total in either direction). The ratio of GC to CG neighbours is generally 10:1 in human DNA and in this repeated sequence the GC to CG ratio seems to be significantly different in that it amounts to 2:1, which is surprisingly similar to the folded viroid sequence recently reported⁷. Also, several regions of symmetry and dyad symmetry occur; for example the base sequence 32-39 is complementary to that of 43-50 (Fig. 3). Dyad symmetry has been seen in control regions in the bacterial genome⁸ and is possibly associated with secondary structure resulting from such dyads. Similarly, the monkey sequences have also been noted to have dyad symmetry⁵ and although these are not in exactly the same positions as in the human sequence, it is interesting that they are maintained in both sequences despite the base changes occurring with evolution. Although all the tandem repeats in this human fragment and other multimer fragments showed other base variations (unpublished data), the constraints on change were remarkable in that the total divergence from the monkey sequence for each of the human variants was always of the order of 35%.

Sequence homology between divergent species may be associated with biologically important molecules, such as DNA coding for histones or ribosomal RNA^{9,10}. Thus, the maintenance of this complex repeated sequence, from monkey to man, suggests that such repeated sequences may have discrete, specific and biologically important functions in the genome. In this context, it is of interest that repeated DNAs with restriction lengths of the order of 174 base pairs have been considered to have a role in the phasing of chromatin in eukaryotic cells¹¹.

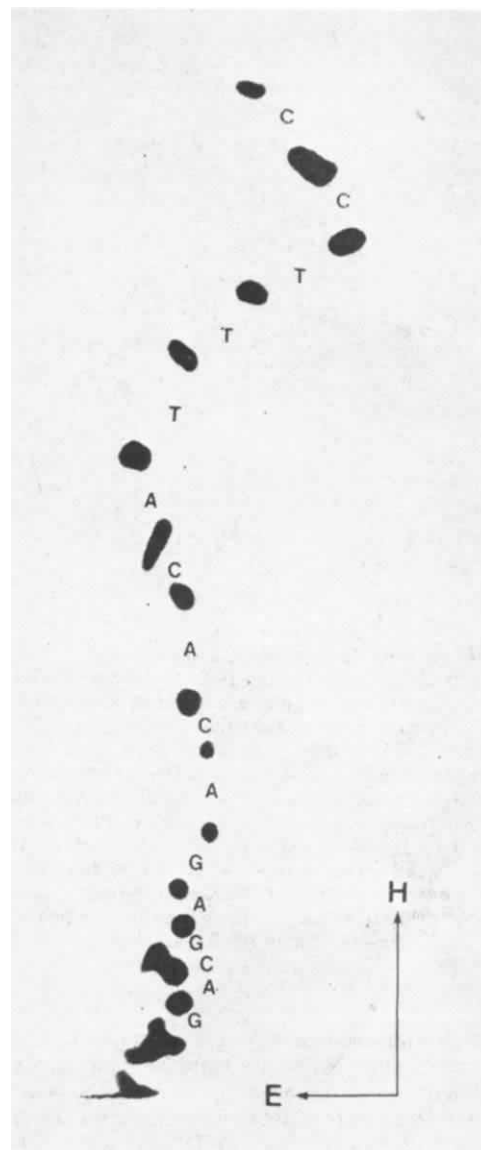


Fig. 2 Isolated 242-base pair fragments corresponding to position 53-294 of the 342-base pair dimer, were labelled at the 53 position with ³²P. Partial snake venom digestion as described² shows single spots for each position corresponding to bases 55-70 in Fig. 3. E is the direction of electrophoresis (first dimension), H is homochromatography as described², in the second dimension.

'Spacer' sequences compatible with the lengths or tandem lengths seen here are also found between coding regions of eukaryotic genes^{10,12}, and such spacers have been considered to be involved in the control, or post-transcriptional splicing, of coding translated gene regions. Similarly, such sequences are also of interest in the processing and integration of viral information¹³. A role for the human complex repeats reported here in similar processes may also be considered. Although these repeated sequences have been found to be highly clustered on several centromeres of the human genome¹⁴, their location in small numbers in other noncentromeric regions of the genome, possibly with slightly different effects at those regions, cannot be ruled out, as chromosomal hybridisation is insufficiently sensitive for a convincing demonstration of small numbers of these molecules.

We have previously considered that the clustering of human complex repeated DNAs on certain human chromosomes may

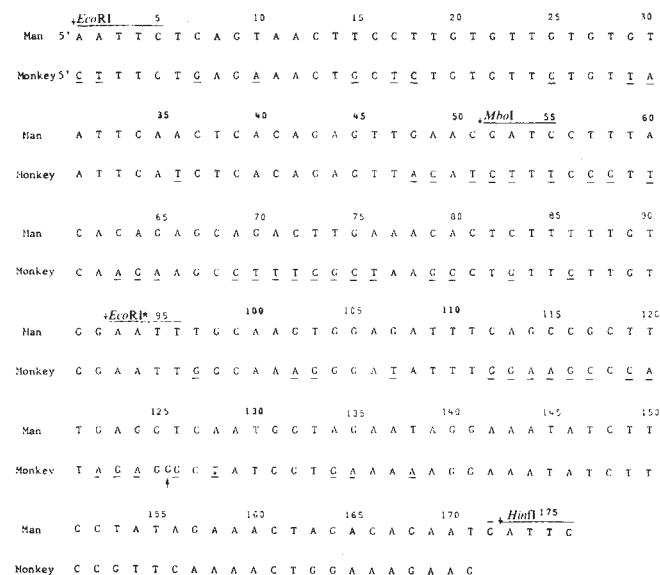


Fig. 3 The nucleotide sequence of a single strand of the tandem repeat (first 171 bases) from human 342-base pair *EcoRI* DNA fragment, compared with equivalent strand of reiterated monkey DNA⁵. Restriction endonuclease sites on the human DNA are shown. The monkey nucleotides which differ from the human sequence are underlined. In the monkey DNA, note also the extra G between nucleotides 125 and 126 (arrow). Dyad symmetries > 5 base pairs occur in the human sequence at bases 26–31 and 59–64; 32–39 and 43–50; 74–78 and 109–113; 108–112 and 141–145; 137–143 and 149–155; 2–6 and 134–138; 14–18 and 139–143. A palindromic sequence occurs in 19–28. Note the base change in the *HinfI* site (at 172) as the sequence repeats, making the site inaccessible to *EcoRI* digestion.

aid the definition or organisation of groups or domains of chromosomes¹⁴, and possibly also in the evolution, association and function of such chromosome groups. In this context it is of interest that chromosome hybridisation studies using various simple sequence satellite human DNAs, and also banded chromosome morphology, have been used to define different human chromosome domains and to infer their evolutionary relationship to specific chromosomes in other groups of primates¹⁵. The secondary structure given by the dyad symmetries (fold-back regions in the molecule) could possibly be related to the proposed unique centromeric structures as well as to less tightly wound regions between chromosome bands¹⁶. Such fold-back regions might allow the association of specific proteins, and thus aid in changing local chromosome configuration and condensation.

This work was supported by NIH grant Ca. 15044. L.M. is the recipient of RCDA award 5-K04-NS-00101.

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Aldolase-catalysed inactivation of glyceraldehyde-3-phosphate dehydrogenase

ALDOLASE is known to catalyse its own inactivation in the presence of substrate and electron acceptors¹. The underlying mechanism is that in the presence of electron acceptors aldolase catalyses the oxidation of dihydroxyacetone phosphate to hydroxypyruvaldehyde phosphate^{2,3} and this highly reactive nascent α -dicarbonyl reacts *in situ* with an active-centre residue⁴. Inactivation of aldolase is entirely due to reaction *in situ* of nascent hydroxypyruvaldehyde phosphate, for hydration and dilution of released hydroxypyruvaldehyde phosphate practically abolishes its contribution to inactivation⁴. As the inactivating agent loses reactivity on diffusion into solvent⁴, its action is restricted to the enzyme molecule that has produced it; other molecules of aldolase or other enzymes (transaldolase, transketolase) are not inactivated by aldolase-generated reagent¹. We report here that oxidative cycles of aldolase cause the inactivation of glyceraldehyde-3-phosphate dehydrogenase (GAPD), suggesting a direct transfer of the inactivating agent between the two enzymes. Kinetic evidence for an interaction between aldolase and GAPD has been presented⁵.

The effect of oxidative cycles of aldolase on the activity of GAPD is shown in Fig. 1. Concomitant with the aldolase-catalysed formation of hydroxypyruvaldehyde phosphate, GAPD is rapidly inactivated. However, this rapid inactivation is not observed when any component necessary for hydroxypyruvaldehyde phosphate synthesis is omitted from the reaction mixture or when GAPD is added after the completion of oxidative cycles. The inactivating agent thus has the same properties as the agent responsible for aldolase suicide¹ in being a transiently reactive nascent product of the oxidative cycle of aldolase. The slow inactivation observed when the enzyme is incubated with hexacyanoferrate in the absence of aldolase or fructose-1,6-bisphosphate is due to oxidation of some essential amino acid side-chains, as no inactivation occurs when the oxidant is omitted from the reaction mixture.

The efficiency of transferring the nascent inactivating agent from aldolase to GAPD, that is, the probability of GAPD inactivation per oxidative cycle, was studied at constant aldolase concentrations as a function of increasing GAPD concentration. As shown in Fig. 2, the efficiency of GAPD inactivation (mol GAPD subunit inactivated per mol hexacyanoferrate reduced) is a hyperbolic function of GAPD concentration. The most plausible interpretation of this curve is that at high GAPD concentrations practically all the available sites on aldolase capable of directly transferring the inactivating agent are exhausted because all aldolase molecules are complexed with GAPD. In agreement with the assumption that the curves in Fig. 2 reflect complex formation, the three sets of data can be described by theoretical curves with the same apparent dissociation constant ($K_d = 10^{-5}$ M). Maximum efficiency of transfer corresponds to efficiency of transfer within the complex and

thus its value is independent of aldolase concentration (Fig. 2, inset). The data in Fig. 2 show that when all aldolase molecules are complexed 0.08 mol GAPD subunit is inactivated per mol hexacyanoferrate reduced. As 2 mol of hexacyanoferrate are reduced per mol hydroxypyruvaldehyde phosphate formed², 0.16 mol GAPD subunit is inactivated per oxidative cycle; that is, one out of six cycles brings about inactivation. It may be noted that the probability of GAPD inactivation is thus an order of

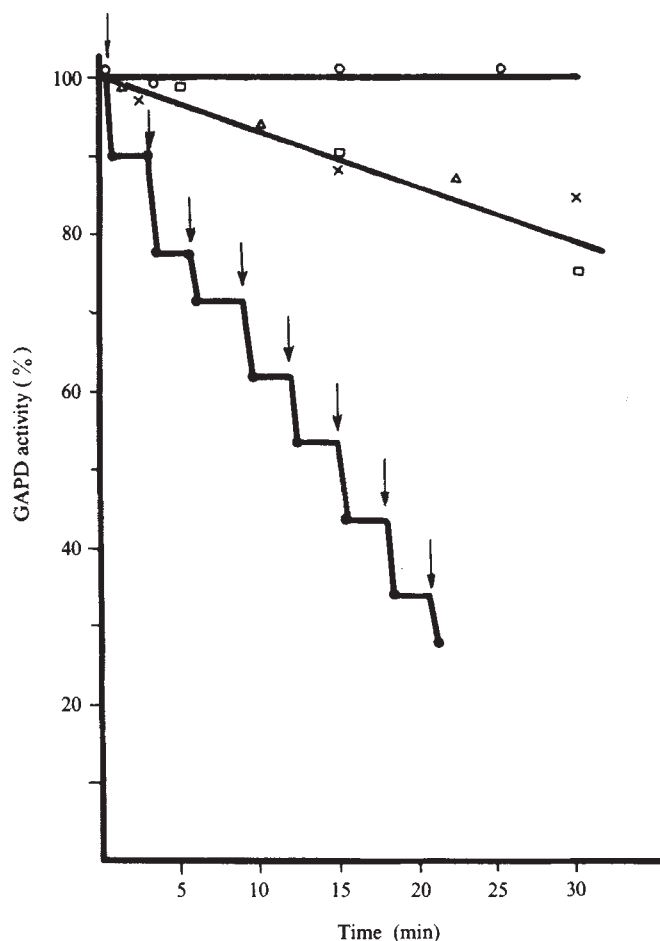


Fig. 1 Inactivation of glyceraldehyde-3-phosphate dehydrogenase during oxidative cycles of aldolase. Rabbit muscle GAPD (2×10^{-5} M) was incubated in the presence of rabbit muscle aldolase (10^{-4} M), fructose-1,6-bisphosphate (5 mM) and repeated doses of hexacyanoferrate (III) in 0.1 M triethanolamine-HCl buffer, pH 7.5, at 20°C, (●). Hexacyanoferrate (III) doses (1.0 mM) were reduced immediately (not shown), and after assaying GAPD activity a new dose was added to the reaction medium at the times indicated by the arrows. In control experiments hexacyanoferrate (III) (○), aldolase (×) or fructose-1,6-bisphosphate (Δ) were omitted from the medium or GAPD was added to the reaction mixture only after the reduction of 8 mM hexacyanoferrate (III) (□).

magnitude higher than that of aldolase self-inactivation, for in the case of aldolase only one out of 70 cycles is accompanied by aldolase inactivation⁴. This difference can probably be ascribed to a more favourable orientation of the inactivating agent in reacting with a GAPD residue than with an aldolase residue, because in identical experimental conditions aldolase and GAPD are inactivated by synthetic hydroxypyruvaldehyde phosphate at similar rates (0.22 and 0.25 $\text{mol}^{-1}\text{min}^{-1}$, respectively).

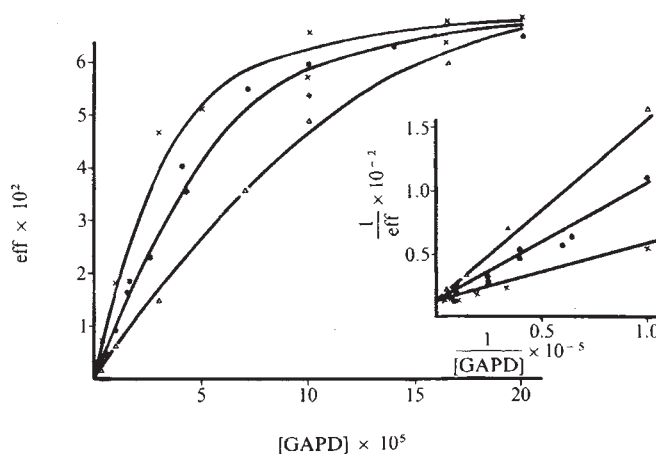


Fig. 2 Efficiency of aldolase-catalysed GAPD inactivation as a function of GAPD concentration. Aldolase at concentrations of 3×10^{-5} M (×), 6×10^{-5} M (●) and 12×10^{-5} M (Δ) was mixed with increasing concentrations of GAPD in the presence of fructose-1,6-bisphosphate (5–15 mM). Repeated doses of hexacyanoferrate (III) were added to each mixture and loss of GAPD activity was determined. Efficiency (eff) of GAPD inactivation (mol GAPD subunit inactivated per mol hexacyanoferrate reduced) is plotted against GAPD concentration. The solid lines are theoretical curves calculated on the basis of $K_d = 10^{-5}$ M. The inset shows the double reciprocal plot of the data.

Affinity labels, suicide inhibitors whose active-site directed action is based on the binding and/or kinetic specificity of the target enzyme⁶, are extremely useful in identifying residues in or near the active centre of enzymes, as the reactive molecule accumulated or generated in the active site reacts preferentially or exclusively with nearby amino acids. The rationale of the present investigation is that, if amino acid side-chains of another protein molecule, through complex formation with the target enzyme, are brought within the action radius of the active-site directed reagent, covalent labelling of these residues might also occur. Accordingly, site-directed reagents may also be used to demonstrate protein-protein interactions. A similar rationale—the use of covalently bound photoaffinity labels—has been exploited for the demonstration of an interaction between cytochrome c and cytochrome c oxidase^{7–9}, and association of chymotrypsin molecules¹⁰.

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matters arising

Non-biogenic fixed nitrogen in Antarctic surface waters

PARKER *ET AL.*¹ have reported measurements of the fixed nitrogen content of Antarctic ice core samples, and calculate an annual input to the Antarctic ice sheet of 27×10^6 kg(N). Following Wilson and House², Parker *et al.*¹ suggest auroral activity as one possible source of this fixed nitrogen.

Auroral activity may produce $\sim 4 \times 10^9$ kg (N) per yr during sunspot maximum (I assume 100 h of IBC Class II aurora per yr, extending over both polar caps, of total area 7×10^7 km² (ref. 3), a production of one ion pair per 35 eV energy input, and 1.3 NO molecules produced per ion pair⁴). This auroral nitrogen fixation occurs in the 70–90 km altitude range, and I do not consider it likely that a large fraction of the nitrogen fixed here will reach the surface because the photochemical lifetime of NO above 40–50 km is significantly shorter than the mean transport time down to this altitude (several days compared with a few weeks). At altitudes below 40 km or so NO has a very long photochemical lifetime, of the order of years, comparable to the mean transport time for an inert tracer to move from these altitudes down to sea level. This long transport time implies a uniform worldwide distribution, and the Antarctic represents approximately 3% of the total surface area of the Earth, so that in the absence of photochemical destruction the auroral source would deposit $\sim 8 \times 10^8$ kg(N) per yr on Antarctica. I estimate (but cannot prove without a detailed calculation) that photochemical destruction may reduce this by perhaps two orders of magnitude on account of the ratio of photochemical to transport times in the lower mesosphere.

However, galactic cosmic ray ionisation produces $\sim 2 \times 10^7$ kg(N) per yr in NO molecules over each polar cap, principally at altitudes between 10 and 20 km. Formation of nitric acid and subsequent precipitation scavenging provides a mechanism for bringing this fixed nitrogen to the ground. Admittedly, the polar caps in this context extend well beyond the Antarctic continent³, but the atmospheric circulation associated with the meteorological polar cell will bring much of this material down to the ground near Antarctica. Galactic cosmic ray ionisation has a maximum at sunspot minimum, so that 11-yr modulation of variation by a factor of two would be expected.

I thank Mr W. W. Berning and Drs M. G. Heaps, R. C. Oliver, G. C. Reid, J. Romick, and J. M. Zinn for discussions, and the High Altitude Pollution Program

of the US Federal Aviation Administration for financial support (contract DOT-FA-77WA3965).

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PARKER *ET AL.* recently claimed that inorganic nitrogen-laden ice originating on the Antarctic continent might provide a major contribution to the nitrogen budget for the epipelagic zone of the Southern Ocean located south of the Antarctic convergence¹. They calculated annual inputs via continental ice for NO₃-N and NH₄-N of 2.73×10^4 tonnes and 1.88×10^4 tonnes, respectively. Assuming an average concentration for surface NO₃-N of $2.7 \mu\text{g l}^{-1}$ and an upper mixed layer volume of $50 \text{ m} \times 14 \times 10^6 \text{ km}^2 = 7 \times 10^{17} \text{ l}$, they estimated the total NO₃-N in the upper mixed layer at 19×10^5 tonnes, and concluded that 7% of this total is injected annually by the melting of Antarctic continental ice.

This estimated input of non-biogenic fixed NO₃-N, 2.73×10^4 tonnes $\div 19 \times 10^5$ tonnes, is mathematically equivalent to about 1% of the estimated total NO₃-N in the upper mixed reservoir, however, rather than 7% as reported by Parker *et al.*¹. While it might be argued that an input of non-biogenic fixed NO₃-N which is 1% of the epipelagic reservoir standing total should not be dismissed from budget calculations, I suggest that the total NO₃-N in the reservoir has been underestimated in the calculation of Parker *et al.*, and that the input of NO₃-N from continental ice is more nearly equivalent to about 0.02% of the upper mixed layer total and can be ignored.

The estimate made by Parker *et al.* of $2.7 \mu\text{g l}^{-1}$ (range 0.02 – $21.4 \mu\text{g l}^{-1}$) for mean NO₃-N concentration of Antarctic circumpolar surface water was derived from summary data tabulated as part of the Antarctic Map Folio series². A comparison with the source nutrient data for this series^{3,4} and with a second tabulation of the same data⁵, however, demonstrates that the units of nutrient concentration were incorrectly given in the Folio series as $\mu\text{g l}^{-1}$ when they, in fact, represent $\mu\text{g-atm l}^{-1}$ concentrations. Assuming a mean surface NO₃-N concentration of $12 \mu\text{g-atm l}^{-1}$ ($168 \mu\text{g NO}_3\text{-N l}^{-1}$) then, the amount of NO₃-N in the epipelagic Antarctic region can be recalculated at $7 \times 10^{17} \text{ l} \times 168 \mu\text{g l}^{-1} \div 10^{12} \mu\text{g tonne}^{-1} = 1.18 \times 10^8$ tonnes. The estimated annual input of NO₃-N from

continental ice¹ is thus 0.02%, or a minor component of the estimated reservoir standing total.

Parker *et al.* did not undertake a similar calculation for the relative contribution of non-biogenic fixed NH₄-N derived from continental ice, as little information on epipelagic NH₄-N levels in the Southern Ocean is available in the literature¹. Recently, I measured water column NH₄-N concentrations at 16 oceanographic stations in the Ross Sea and south of the Antarctic convergence. NH₄-N was assayed by a modified Solorzano phenol-hypochlorite method⁶ aboard USCGC Glacier during December 1977–January 1978. Water samples taken in 5-l Niskin bottles were filtered through $0.45 \mu\text{m}$ GF/A filters, fixed with phenol alcohol, refrigerated, and analysed within 24–48 h of collection, using a 10-cm path length in a Bausch & Lomb model 710 spectrophotometer. Surface NH₄-N concentrations in ice-free areas averaged $0.1 \mu\text{g-atm l}^{-1}$ (range <0.1 – 0.3), with higher concentrations of NH₄-N in subsurface maxima⁷. Subsurface NH₄-N maxima generally occurred deeper than 50 m, and I here assume that $0.1 \mu\text{g-atm l}^{-1}$ is representative of NH₄-N concentrations in the upper mixed layer. Using Parker *et al.*'s estimate of $7 \times 10^{17} \text{ l}$ for volume of the upper mixed layer¹, I calculate a reservoir total for NH₄-N of $7 \times 10^{16} \mu\text{g-atm}$, or 1.2×10^6 tonnes. Thus, the estimated annual input of non-biogenic fixed NH₄-N from continental ice¹ is mathematically equivalent to about 2% of my estimated reservoir total.

While the input of NH₄-N derived from continental ice, as a percentage of its reservoir total, is proportionally larger than the input of NO₃-N derived from continental ice as a percentage of its reservoir total, the former is apparently much less than the biogenic input of NH₄-N. For example, a conservative estimate is that 500 million tonnes of krill (*Euphausia superba*) are produced annually in the Southern Ocean^{8,9}. Ammonia is the primary nitrogenous waste of planktonic crustacea^{10–14}, and shipboard measurements of NH₄-N excretion by individual Antarctic euphausiids⁷ suggest that this amount of krill alone might excrete approximately $1.1 \times 10^{16} \mu\text{g-atm NH}_4\text{-N d}^{-1}$.

Daily NH₄-N excretion by krill, then, may represent about 8 times the annual non-biogenic input of NH₄-N from continental ice¹, or about 16% of my estimated epipelagic reservoir NH₄-N total. If one assumes that krill, which live primarily in epipelagic Antarctic waters as adults^{8,9,15}, excrete their NH₄-N wastes exclusively there, it follows that NH₄-N in

surface waters should turn over at least once per week.

In summary, it is likely that the input of non-biogenic $\text{NO}_3\text{-N}$ and $\text{NH}_4\text{-N}$ derived from continental ice is probably insignificant in the overall nitrogen budget for epipelagic Antarctic seas although enrichment from melting ice may be locally important. It is further likely that the biogenic input of $\text{NH}_4\text{-N}$ (from zooplankton and nekton excretion) far outweighs $\text{NH}_4\text{-N}$ inputs from continental ice, and the calculated rapid turnover rate for $\text{NH}_4\text{-N}$ suggests that ammonium could conceivably limit the rate of primary production in the Southern Ocean.

I thank the officers and crew of USCGC Glacier for logistical support of oceanographic research in the Ross Sea 1977–78. Research was supported by a grant to S. Z. El-Sayed from the Division of Polar Programs, NSF (DPP76-80738-A01).

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PARKER ET AL. REPLY—We too are concerned that data may be too limited and assumptions too many to draw fixed conclusions. We agree with Biggs' calculations for both NO_3^- and NH_4^+ contributions to the Southern Ocean. However, the calculations were based on mean values, assuming uniform mixing to a depth of 50 m. Yet icebergs are not distributed uniformly through time and space, nor are their nitrogenous contents uniform. Regretting our selection of El-Sayed's published data which cited $\mu\text{g l}^{-1}$ rather than the correct $\mu\text{g-atm l}^{-1}$ for $\text{NO}_3\text{-N}$, we note that the ranges of concentrations vary widely such that icebergs might well be found in ocean waters well below the concentration levels reflected by the mean ice values. Since our report, we have completed over 1,000 analyses of glacial ice and find values ranging to $525 \mu\text{g NO}_3\text{-N l}^{-1}$ ($\sim 37 \mu\text{g-atm}$) and to $260 \mu\text{g NH}_4\text{-N l}^{-1}$ ($\sim 19 \mu\text{g-atm}$). Also Robe¹ has estimated that icebergs melt primarily in the upper 20 m of water. These points could bring about

higher fixed nitrogen contents in surface water at least locally. Indeed, we reported in a note added in proof in our original paper that we found icebergs with "concentrations near our estimates and that a concentration gradient occurs, descending with increasing distance from an iceberg". Chlorophyll content was several times higher in surface water adjacent to that same iceberg when compared with water approximately 2.0 km downwind from the same iceberg. These studies were preliminary and were conducted in McMurdo Sound involving icebergs which probably originated from the Ross Ice Shelf, the nitrogenous composition of which is unknown. We therefore assert that the overall question of whether nutrients from icebergs or ice shelves play a major part in the productivity of the Southern Ocean remains moot, and that the probability of icebergs enhancing productivity of surface waters locally or seasonally on a wider scale cannot be dispelled from the available data, assumptions and calculations. We plan a more extensive test of our thesis during the coming austral summer.

With respect to Bauer's comments, we also agree with the calculations for lack of other information to contradict the numerous assumptions made. However, we question whether NO can be destroyed photochemically in polar regions during winters when solar radiation is essentially absent for periods of up to several months. Assuming the maximum angle of 23.5° for the Sun at winter solstice, we calculate that solar radiation will be absent to an altitude of >100 km for more than 5 months. More specifically, disregarding the minor components of scattering and refraction, we calculate the following approximate altitudes above sea level at the geographic poles which will not receive direct sunlight: at the winter solstice, 575 km; ± 30 d from winter solstice, 439 km; ± 45 d from winter solstice, 300 km; ± 60 d from winter solstice, 156 km; ± 75 d from winter solstice, 46 km; ± 90 d from winter solstice, 0.278 km. These values are only slightly lower when we recalculate values using the upper periphery of the Sun instead of the centre.

Therefore, if Bauer's mean transport time for NO from 70–90 km to 40–50 km is correctly estimated at a few weeks, there is ample time during the polar night for aurora-fixed nitrogen to reach the Earth's surface without photodestruction. Even with early spring and late autumn, much of the UV radiation will be removed by the thick layer of ozone through which the Sun's rays must penetrate to reach the polar region. We assert that, based on our data for a 100-m South Pole core, the periodic fluctuations in NO_3^- concentrations cannot be simply explained by a model using galactic cosmic rays as a major mechanism for explaining the source of NO_3^- in Antarctic snow.

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EL-SAYED COMMENTS—The above authors quoted data included in my Tables 1 and 2 which appeared in Balech *et al.*¹. Unfortunately the units of nutrient concentrations were incorrectly printed as $\mu\text{g l}^{-1}$. However, the maps in that folio showing surface phosphates and silicates (but not the nitrates and nitrites) were correctly given in $\mu\text{g-atm l}^{-1}$. This table appeared in ref. 2 with the concentration units given in $\mu\text{g-atm l}^{-1}$. Except for the misprint in this folio, all my nutrient data are always given in $\mu\text{g-atm l}^{-1}$ (see refs 3–6, 7). Reference to these other publications would immediately have pointed to the misprints in the units given in ref. 1.

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Carbon isotope fractionation in biological material

STABLE, rather than radioactive, tracers are being used in metabolic studies, and Lyon and Baxter¹ have reported "natural baseline" carbon isotope ratios from human tissue samples. The use of natural isotope ratios is also an area of active research interest because of the ecological and palaeoecological significance of carbon isotope discrimination in higher plant photosynthesis². These botanical and ecological studies provide information which may clarify and illuminate some aspects of the above report.

The $^{13}\text{C}/^{12}\text{C}$ ratios, expressed as $\delta^{13}\text{C}$ against PDB, for land plants generally range from -8% to -32% (ref. 3) rather than the -24% to -29% reported¹, and are distinctly bimodal near -13% and -27% . These two modal clusters represent the two major photosynthetic systems (C_4 and C_3 , respectively) which discriminate differently, and diagnostically, against the carbon isotopes. The mechanism for this discrimination is quite

well understood as is the basis for the differential. Most temperate-zone agriculture crops are of the C_3 type and possess a $\delta^{13}C$ near -27% . Many tropical grasses (including maize, sorghum and millets) and relatively few dicots are C_4 and possess a $\delta^{13}C$ near -13% . The mean human tissue values of around -23% reported by Lyon and Baxter may simply reflect a dietary carbon source which consists mainly of C_3 plants. Thus, the human samples in the above study were probably of Northern European derivation. Also, I would expect subject variability to be high, as the ratio will vary in a manner related to the dietary carbon. Presumably, this would range from near -13% for diets derived from C_4 plants to near -27% for food from C_3 plants.

The real question is whether there is secondary fractionation following the initial carboxylation of CO_2 . There is some direct evidence for this in plant tissues and biochemical fractions^{3,4} and indirect evidence in animals³. This additional discrimination, however, is small and seems most important in lipid synthesis where further discrimination against ^{13}C occurs⁵. Recent experimental data suggest that derived organic matter reflects dietary $\delta^{13}C$ but tissues and biochemical fractions differ in $\delta^{13}C$ values⁷.

As the organic matter of secondary production will reflect the dietary values, a further complication is introduced. Each tissue and biochemical fraction may also have an isotopic 'memory'. This will be a function of the $\delta^{13}C$ of the carbon at the time of synthesis, the $\delta^{13}C$ values of subsequent foodstuffs, and the biochemical turnover rate of the tissue or fraction. The persistence of this memory as well as the magnitude of secondary discrimination for various biochemical fractions must be established before results of certain ecological⁶ and perhaps metabolic studies can be considered reliable.

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Prevalence of male brood care in teleosts

DAWKINS AND CARLISLE¹ have suggested that male brood care among teleosts arises from the female's ability to spawn first and then desert her consort, placing him in Trivers' 'cruel bind'². The vulnerability of sperm to diffusion ostensibly bars this option to males. A comparative analysis of teleost reproductive biology lends little support to this argument, however. Of the 38 teleost

families with at least one parental species for which the spawning behaviour is known, 23 (60.5%) are characterised by simultaneous expulsion of gametes³⁻⁵. As neither sex enjoys a temporal advantage in these cases, the desertion argument should predict a random distribution of paternal, maternal and biparental care. On the contrary, paternal care characterises 16 of these families^{6,7}, a significantly nonrandom proportion ($\chi^2 = 19.00$, d.f. = 2, $P < 0.005$).

The desertion argument predicts paternal care in species in which oviposition and ejaculation are well separated in time. In the cave-spawning cichlids, the best known group of fish of this type, brood care ranges from biparental to fully maternal, however^{4,8-11}. Maternal mouthbrooding cichlids have the most clearly sequential spawning pattern known¹². Yet the female commits herself irrevocably to a parental role before the eggs are even fertilised^{8,12}.

Finally, maternal care is predicted where the male spawns first. This pattern occurs in the maternal mouthbrooding cichlid *Sarotherodon macrochir*¹³ and the paternal custodial goby *Bathygobius soporator*¹⁴. In neither case has this behaviour led to a departure from the genus-typical pattern of brood care.

The data suggest that factors other than the sequence of gamete deposition may account for the prevalence of paternal care in fishes. I propose an alternative model, based on (1) the limited availability of suitable spawning sites¹⁵⁻¹⁹, which makes sequestration of a breeding territory selectively advantageous for many teleosts with demersal eggs⁵⁻⁷, and (2) the differential energetic unit-cost of producing ova and spermatozoa^{2,20,21}, which makes sequestration less profitable for females than males as a means of maximising reproductive output. Extending the reasoning of Maynard-Smith²² and of Dawkins and Carlisle¹ to include access to any resource necessary for reproductive success, male desertion after spawning would be selectively advantageous only where there is high probability of securing another breeding site. Where these are limiting, this eventuality is unlikely. Persistence is thus favoured because an immediate payoff to a territorial male is the continued opportunity to spawn.

Defending an area against intrusion incidentally affords some protection to eggs within its boundaries^{23,24}. Thus in any system characterised by nest disturbance or egg predation, a difference in fry production between guarded and unguarded sites would strongly favour post-spawning territorial behaviour even if other factors reduce the likelihood of sequential polygyny. Its evolution would be facilitated because it would not require incorporation of new motor patterns or endocrine control systems into the reproductive repertoire. Only prolongation of elements already involved in prior

site sequestration would be necessary. Given a commitment to spawn defence, selection could then operate to produce complex brood care, following the schema outlined by Barlow²⁵.

Within the framework of this model, biparental brood care is a derived state, its evolution favoured by environmental factors such as extreme scarcity of spawning sites^{8,18,26} or severe spawn predation^{10,18,27}, which make switching available energy from egg production to behavioural activities advantageous for females²⁵. Maternal care could be derived from a biparental precursor, with male desertion explicable in terms of the modified parental investment model proposed by Dawkins and Carlisle¹.

To conclude, the limiting nature of spawning sites and the differential bioenergetics of maturing eggs and sperm, rather than the mechanics of spawning with internal fertilisation, seem to account for the prevalence of male brood care among teleosts.

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DAWKINS REPLIES—I agree with Loisel that the comparative data do not support the small part of our paper which was concerned with male brood care among teleosts. A similar conclusion is reached in a recent exhaustive review of the literature on paternal care¹. The primary purpose of our paper, to expose the 'Concorde fallacy', remains valid.

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reviews

Stellar atmospheres

Bernard Pagel

Stellar Atmospheres. (Second edition). By D. Mihalas. Pp.631. (Freeman; San Francisco, 1978.) \$24.95.

THE 'atmosphere' of a star is a thin surface layer that is of importance for two fundamental reasons: (a) it is the part that can be seen from outside and thus gives information on the fundamental stellar parameters of effective temperature, surface gravity and chemical composition which in turn are related to internal structure and evolution; and (b) it plays an essential part in the internal economy of the star by radiating away the power generated by nuclear processes in its interior. In addition, stellar surface layers are the seat of a wide range of interesting physical effects, including solar activity (which undoubtedly has counterparts in other stars) and the presence of kinetically hot, tenuous outer regions which interact with the interstellar medium through continuous mass loss and other effects.

The classical theory of stellar atmospheres, which developed from pioneering studies by W. H. McCrea in the early 1930s through R. Wildt's discovery of the dominant role of H^- in solar-type stars in 1938 to the moderately accurate stellar abundance analyses of the 1950s and 1960s, rested on a number of simplifying assumptions: radiative and hydrostatic equilibrium and the approximation of local thermodynamic equilibrium (LTE). The latter, first introduced mainly for computational convenience, has proved remarkably successful in accounting for the continuous spectrum and most absorption lines (though not emission lines) from the Sun and many other stars, and can indeed be quantitatively justified for the visual continuum of the Sun and all but the hottest stars along the main sequence. It was strongly challenged in the 1950s and 1960s, however, on grounds that were quite justified in general terms; but no improvement over LTE interpretations of absorption lines was achieved until the development of really accurate non-LTE theories, for which the author of the present book was largely responsible, in the 1960s. It is largely as a result of his deep insight and his expertise in computational methods

that we now have a viable non-LTE theory of stellar atmospheres which shows that, in the hottest stars and stars of high luminosity, the more general assumption of a local steady state indeed leads to a much more accurate interpretation of stellar continua and line profiles; in solar-type stars however, the departures from LTE are large for emission lines and for certain lines in the infra-red, but are quite small for the total intensities of other lines.

The first edition of this book, which appeared in 1970, was a masterly exposition of the physics and mathematical methods involved in both the LTE and the non-LTE theories of stellar atmospheres, with special emphasis on the continua and absorption line spectra of the hotter stars. The present edition has been considerably enlarged to include newer developments, particularly in the study of extended atmospheres and moving media, and treats the whole problem from a more general point of view, including the hydrodynamics of stellar winds, as well

as discussing the radiative transfer problems that formed the main emphasis in the first edition. Another refinement is the discussion of departures from complete redistribution in frequency of scattered photons; "partial redistribution" (which approximates the concept referred to as "coherent scattering" in the older literature) is essential in the discussion of such phenomena as the chromospheric emission cores of the broad Ca^{2+} and Mg^{2+} absorption lines in cooler stars, a point which had been overlooked in many of the non-LTE treatments of this effect. Finally, the author has added a number of well chosen problems which serve both to carry the argument along and to impress it on the reader's mind.

The new edition of this outstanding book is thoroughly up to date and is in every way a classic worthy of its predecessor. □

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Susceptibility to environmental pollutants

Pollutants and High-Risk Groups: The Biological Basis of Increased Human Susceptibility to Environmental and Occupational Pollutants. By E. J. Calabrese. Pp.266. (Wiley: New York and Chichester, UK, 1978.) £13.75.

It has been recognised by toxicologists for a long time that sensitivity to certain toxic agents depends on sex, age, nutrition, circadian rhythms and pre-existing morphological and biochemical disorders. Experience has also proved the value of medical examinations in preventing the occupational exposure of those who might be susceptible to chemical and physical hazards. This book, being a comprehensive review of known and hypothetical 'high risk' groups, helps one to appreciate that increased susceptibility to environmental pollutants is a widespread problem which must be kept in mind in the development of environmental health standards. One chapter deals with age, pregnancy and circadian rhythms; other chapters deal

with genetic disorders, nutritional factors and diseases. There is a list of screening tests and a general discussion on the public health implications of high risk groups. Approximately 900 publications are referenced in alphabetical order and with title.

The pioneer enthusiasm of the author is evidenced by a coverage which oversteps the legitimate boundary of the subject. Thus, the reader is assured that due to developmental and circadian weak points in our life cycle and daily lives, every individual at some point is at increased risk. This overzealous approach leads to statements like: "These programs (that is the hot-lunch program for the elderly) are needed in the inner cities, where diets are notoriously poor and environmental pollutant levels (eg ozone) are usually high". Hypotheses are sometimes presented as facts and inadequate proof is not criticised. By linking a report on 20 cases of CO poisoning in pregnant women to increased CO production in pregnancy, the author gives

the impression that endogenous CO production predisposes pregnant women to CO intoxication.

Another weakness of the book is probably due to the policy of the publisher: to widen the possible circle of readers as far as possible by giving some background material. Information like "In man, the kidneys are bean-shaped structures about 4 in long" and that "Renal arteries and veins are supplied to the kidneys" somewhat underestimate the intelligence of the non-science graduate readers, who are

supposed to understand quite complex biochemical processes which are explained at a significantly higher level.

However, compared with the valuable information and pioneer spirit, this criticism is only of minor significance and it would be wrong if this diverted people interested in environmental health from reading the book and thinking about the problems.

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along the gut and into the genital ridge, and the influence of the somatic cells of the gonad on germ cell maturation and secondary sexual organ development are discussed in articles by Heath, O, and Donahoe and Swann.

The remaining articles move on to the wider arena of morphogenesis in the mammalian embryo. Looking at the incredible scanning electron micrographs of neurula stage embryos presented in this volume one can only admire scientists like Snow, and Morriss and Thorogood, who have begun the painstaking task of analysing the forces responsible for generating such complex, three-dimensional shapes. One of the most stimulating ideas in this field, put forward recently by Snow and discussed in his review, is that all the tissues of the mammalian foetus are derived from a small group of rapidly dividing cells, or proliferation centre, in the early epiblast. Testing this hypothesis, and following clonal growth and cell migration, would be much easier if there were a genetic marker in the mouse detectable intracellularly early in development. As it is, the final article by West reviews the information obtained so far using chimaeras between mouse embryos differing in genetic markers expressed late in development.

The book is well produced, although the type is too small for comfort, and the articles are well referenced.

Brigid Hogan

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Development in mammals

Developments in Mammals. Vol. 3. Edited by M. H. Johnson. Pp.472. (North-Holland: Amsterdam, New York and Oxford, 1978.) \$61; Df.140.

In many respects this third volume of *Development of Mammals* is the best so far, offering several excellent reviews of topics which have only recently undergone a burst of stimulating activity. On the other hand, some of the reviews are not without doses of repetition and excessive speculation, and would have benefited from a firmer editorial hand. In the preface, Martin Johnson tells us that he encouraged his authors "to develop new ideas and to be provocative", but sometimes the provocation is of the wrong kind! All in all, however, this is a good volume, and one to which developmental biologists of both the molecular biology and pattern formation schools may turn with interest.

Appropriately, the volume begins with two articles by Meizel and by Green on the act that starts it all—fertilisation—or, rather, on the preview to Act One, in which the sperm undergoes the acrosome reaction. The acrosome is a giant secretory granule lying near the sperm tip, and the membrane of the acrosome fuses with the overlying plasma membrane so that the store of hydrolytic enzymes is exposed. The exact stimulus for the reaction, the mechanism of membrane fusion, and the function of the enzymes are all unknown, and the two articles debate the available evidence.

Recent work on monoclonal antibodies reacting with murine teratocarcinoma cells and early mouse embryos has made the review by Pratt on membrane lipids of embryos a timely contribution. At least one, and probably several, of these monoclonal antibodies recognises a glycolipid, and not a few developmental biologists have been embarrassed to discover their ignorance about this class of molecule.

The role of virus-like particles in early embryos is discussed by Daniel and Chilton, and this is followed by an excellent review by Monk of X-chromosome inactivation in female embryos. This topic is one which has undergone a recent flurry of experimentation, and the author combines the new data with speculation in a nice, economical style.

Teratocarcinoma cells receive their fair share of mention in this volume, and Martin tackles the difficulties as well as the advantages of using *in vitro* cultures of these cells as a model for mammalian development. Her appraisal of the results from several laboratories on the fate of teratocarcinoma cells injected into host blastocysts should be required reading for all molecular biologists who now believe that a normal mouse can be generated from teratocarcinoma cells with, as Martin puts it, "a flick of the micro-manipulator".

The origin of the primordial germ cells, their migration from the yolk sac

Cell proliferation and the cell cycle

Cell Cycle Regulation. Edited by J. R. Jeter, I. L. Cameron, G. M. Padilla and A. M. Zimmerman. Pp. 255. (Academic: New York and London, 1978.) \$23; £14.95.

MANY texts on the cell cycle confuse (wisely?) progression through the cell cycle of continuously replicating cells with the control of cell proliferation in growing populations. This book is largely concerned with the regulation of the progression through the cell cycle in cycling cells, and consists of eleven separate contributions. McCarty reviews recent progress in chromatin structure and surveys in a general way the post-synthetic modifications of chromatin proteins. In no case is the precise molecular function of any of these modifications known; although there is recent and exciting progress in the study of acetylation, phos-

phorylation and ADP-ribosylation.

Gurley *et al.* give a detailed description of their work on phosphorylation through the cell cycle of CHO cells. The phosphorylation of histone H1 in G₁, in S and a "superphosphorylation" at the time of mitosis seems to be generally true. They discuss the controversy with Matthews and Bradbury as to whether superphosphorylation is associated with condensed chromosomes, or is a preceding "trigger event".

Krause discusses the variable binding of histones during the cell cycle. Harris brings together information on microtubules, membranes vesicles and calcium to construct an hypothesis for the initiation of mitosis. Gerson reviews the possible role of intracellular pH in the cell cycle. Recent interest in the possible regulatory functions of such parameters as pH and divalent cation availability revives hypotheses of some 30 years ago.

Physarum rears its beautiful heads twice in this book; it is (of course) the ideal eukaryotic organism for cell-cycle

studies because of its natural synchrony. Sauer contributes a splendid review of gene expression in *Physarum* and adds a stimulating hypothesis for transcriptional control in the cell cycle. This is followed by a fine chapter from Howell on the regulation of protein synthesis in *Chlamydomonas*.

There are also chapters on regulation of specific enzyme induction. *Petite* mutation in yeast and, somewhat out of the mainstream, a discussion by Hoffman of epidermal proliferation in lower vertebrates. Finally, the somewhat brief, experimental article by Rao and Sunkara on cell fusion is full of provocative statements and interesting data. (A full critical review of cell fusion data would be very valuable.)

I enjoyed reading this book and commend it to all biology libraries. The editors tell us that it will provide material for a textbook on the cell

cycle. I offer, with due humility, some comments; a unified textbook should of course be more uniform in style. They will, I hope, rectify two crucial omissions even though "cell cycle regulation is too broad a subject for one volume". The kinetics of the cell cycle and of cell proliferation have provided major insights and will continue to do so in the future; they should be discussed. Even more regrettable is the omission of a discussion of cell cycle genetics. The elegant and informative work of Lee Hartwell and Paul Nurse on yeast and even the considerable, preliminary genetic work on animal cells is a major new excitement in cell cycle studies. They are essential to a contemporary discussion of cell proliferation and the cell cycle.

Sydney Shall

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Hemispheric asymmetry

Asymmetrical Function of the Brain. Edited by M. Kinsbourne. Pp.581. (Cambridge University Press: Cambridge and London, 1978). £18.

STRUCTURAL and functional differences between the left and right hemispheres of the human brain have been investigated intensively by experimental psychologists, neurologists and others for the past fifteen years, and such work continues to proliferate. As research on this topic is still very much in progress, no book on hemispheric asymmetry can offer definitive interpretations at the moment; the best that can be hoped for (and it would of course be valuable) is an up-to-date survey of current knowledge and future prospects. Unfortunately, Professor Kinsbourne's book cannot offer this. Many of the chapters were completed several years ago, as is evident from the absence of recent references in their bibliographies, as well as from the fact that preprint versions of some chapters have been in circulation for a number of years. One chapter, indeed, was written in 1968, revised and expanded in 1970, and had a two-page postscript added still later, alluding to some of the work done since then. Clearly, there has for some reason been a delay of some years between the completion of many of the chapters and the appearance of this volume on the booksellers' shelves; and this means that much of the book is already out-of-date.

The fifteen chapters themselves are very uneven in quality. Two are extremely long, occupying nearly half of

the book between them. One of these, 126 pages devoted to research on the relationship between the report of a tachistoscopically exposed row of letters or other items and the position in the row of the reported item or items, scarcely mentions hemispheric asymmetry, concentrates too much on the author's own research, and was completed in 1970, with no subsequent revision. The other long chapter, a 118-page survey by L. J. Harris of sex differences in spatial ability, is excellent: penetrating, scholarly, exhaustive, and an ideal companion piece to the same author's recent survey of sex differences in the growth and use of language. There is a useful chapter by Nebes reviewing the results of split-brain studies; this is an expanded version of his article on this topic in the *Psychological Bulletin*. It suffers from paying too little attention to the fact, pointed out to me by E. K. Warrington, that all of the split-brain patients whose performance has been studied in any detail showed evidence of brain injury at birth; one would therefore expect them to develop abnormal patterns of hemispheric specialisation; thus, serious difficulties arise if one wishes to infer anything from these patients about hemispheric asymmetries in brains which have developed normally.

The editor of the volume has in several previous publications proposed an interpretation of hemispheric asymmetry effects in terms of asymmetry of attention; a subject whose attention is biased towards his left hemisphere will perform better with material presented to this hemisphere (via the right visual hemifield or right ear) than to the other. The occurrence of, for example, right-hemifield superiorities in visual tasks requiring verbal processing is said

to occur because the requirement for verbal processing creates an attentional bias towards the left hemisphere. Conversely, a non-verbal visual task, creating an attentional bias towards the right hemisphere, will show a left-hemifield superiority. A direct way of testing this attentional interpretation of hemispheric asymmetry effects is to arrange matters so that the subject cannot know in advance whether his task will be a verbal or a non-verbal one; under such conditions there cannot be a preparatory bias towards the hemisphere appropriate to the task. It follows from the attentional bias theory that randomly intermingling the two types of task in an unpredictable way will eliminate hemifield differences for both types of task. Such experiments have in fact been carried out: by Geffen, Bradshaw and Nettleton in 1972 (verbal or non-verbal matching of letter pairs), Berlucchi *et al.* in 1974 (letter-matching and face-matching) and Bryden and Allard in 1976 (randomly varying typefaces, some of which favour the right hemisphere and some the left). In all three studies, selective previous preparation of one or other of the hemispheres was impossible; in all three studies, the appropriate hemifield effects nevertheless still occurred. As these three findings would seem to devastate any attentional interpretation of hemispheric asymmetry effects, one would have expected Professor Kinsbourne to have mentioned them when reiterating this interpretation in his contributions to this volume; but he does not do so.

Because so much of this book was written so long ago, much that is of vital importance for an understanding of the brain's asymmetrical functions is simply not dealt with. One neurological example is the Scandinavian work on hemispheric asymmetries in regional cerebral blood flow during verbal activity; one psychological example is the crucial work of Morais and Bertelson on dichotic listening.

Furthermore, in spite of the long delay in the appearance of this book, it shows signs of being very hastily put together. The subject index is absurd. It is three pages long; the entries concerning spatial ability ignore the 118-page chapter on this topic; and there are no entries for handedness, left-handedness (though there is one for paw preference!), reading, writing, faces or sex differences, to mention but a few obvious omissions. There are, however, index entries directing the interested reader to discussions of the Kalahari Bushman, the flute, the bassoon, the 'cello, and the dowel rod.

Max Coltheart

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Nutritional panacea

Fish Protein Concentrate: Panacea for Protein Malnutrition? By E. R. Pariser, M. B. Wallerstein, C. J. Corkery and N. L. Brown. Pp.296. (MIT Press: Cambridge, Massachusetts, and London, 1978.) \$17.50; £12.25.

NUTRITION tends to be a profession beset by fashions, but poor on introspection. Now that a 30-year obsession with a fictitious world protein crisis has ended, it is generally agreed to have been a fiasco, costly in both money and human suffering. And yet there is remarkably little soul-searching about why it occurred, and how future (or current) outbreaks of myth production can best be terminated.

This book is therefore to be welcomed as hopefully the first of a series of studies on how the protein fiasco developed and was sustained. It is a detailed study or one solution to 'the protein gap', that of fish protein concentrate (FPC), how it was invented and used, and why ultimately it failed. The authors deal primarily with FPC in the United States and they show quite convincingly that the development of FPC followed both the technological imperative (that which can be done, must be done) and a political one of supporting the ailing US fish industry, by providing an outlet for unpalatable fish and fish waste. Humanitarianism was the secondary justification, rather than the primary motivation of the project.

But the FPC story is raised from being a dirge about the duplicity of the aid industry, to the stratosphere of high farce, by the injection of a unique element into the story: filth. For the FPC venture failed, not because a rational analysis of the world's problems showed that no protein deficit existed, or even because someone noticed that bland, odourless powders are not acceptable foods. FPC failed because it was deemed filthy.

When the US Bureau of Commercial Fisheries first sponsored FPC, it necessarily applied to the Food and Drugs Administration (FDA) for permission to sell the stuff as food. This permission the FDA denied on the grounds that FPC was filthy, as, being made of defatted whole fish, it contained fish guts, heads and scales. On these grounds they argued that FPC was "a crime against decency". When evidence was presented that this did not mean that FPC was bacterially contaminated, the FDA countered with the view that filth was an aesthetic judgment, not a bacteriological one. When the FDA were reminded by the National Aca-

demy of Sciences that guts of oysters or clams were habitually consumed, they replied that as these guts were acceptable to the public, they were, *ipso facto*, not filthy. As FPC was made from fish that were not usually eaten, by FDA definition, the guts were filthy.

It's Catch-22 of course: that which you don't eat is unacceptable, therefore you can't be offered it to eat, so you don't eat it. Ironically, FPC had never been intended for the US market. Its primary destination was to have been the third world, but they could hardly be given as aid food declared unfit for consumption by the US regulating agency.

Less than \$20,000,000 were squandered on FPC research in the US and despite its importance in President Johnson's loudly proclaimed "War on

Hunger", FPC was inflicted on very few people for very limited periods. By the time the FDA withdrew their objections to FPC after 12 years, the project was scientifically bankrupt and was quietly allowed to decay.

It is a fascinating, if saddening, story, and though I found the authors' style too full of confusing abbreviations and heavy prose, and felt that the inclusion of some unnecessary material, such as technical references on FPC, added only to its length, not its quality, I nevertheless found it a worthwhile book to read.

I enjoyed this book and I recommend it to nutritionists, historians, old-fashioned radicals and fish-eaters alike.

John Rivers

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Social responses to natural hazards

The Environment as Hazard. By I. Burton, R. W. Kates and G. F. White. Pp.240. (Oxford University Press: New York and Oxford, 1978.) Hardback £5; \$11; paperback £2.95; \$6.50.

THERE is a strong tendency in all but the most primitive of societies to see natural disaster largely as a matter of science, and so to view possible responses in terms of the scientific or technological fix. Thus, as recently as 1972 a United Nations conference could conclude that the causes and prevention of natural catastrophes "fall within the province of science and technology" and offer 30 concrete recommendations of which no fewer than 28 called for new scientific investigations. But as Burton and his colleagues point out at length (indeed, it is their basic premise), it takes two sides to make a natural disaster—an unusual natural event and the community affected by it. It is only to be expected, therefore, that in the long term the reconciliation of man to environmental hazards will require social, no less than scientific, action.

If there are any doubts on that score they must disappear with the knowledge that in recent decades the potential for disaster has been increasing, not as a result of any increase in the violence or extent of the Earth's activity but as a consequence of new social mistakes. It is well known that a human settlement with a commitment to a particular location in terms of capital and a sense of identity will defy either natural or man-made threats to remove

it (as San Francisco's response to the earthquake threat shows). What is less well known, however, is that past ignorance has not yet been replaced by current enlightenment. Man is not only creating new hazards by modifying the environment but is forcing new people and old hazards together in fresh areas. In what is now Bangladesh, for example, land reclaimed from the sea by sophisticated engineering and held by agricultural technology has attracted a huge new population into a zone at high risk from cyclones. The great disaster of 1970, in which a cyclone killed more than 250,000 people in Bangladesh, should therefore have surprised no-one.

Under the circumstances a theory, however primitive, of the social responses to natural hazards is far from being an academic luxury. Yet the social aspects of natural disasters and their mitigation have all too often been ignored in the past, even at the level of basic information gathering, no doubt because of the daunting complexity of human behaviour and the near impossibility of expressing it in even remotely 'scientific' terms. In bringing together and ordering for the first time the results already obtained in this astonishingly complex field, Burton and his co-authors have provided a valuable and fascinating account. They would be the first to admit that the subject is still in its very early stages; but though in that sense incomplete, their book is more than sufficiently coherent to offer an excellent starting point for further studies.

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